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# BIOLOGICAL BULLETIN

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**Marine Biological Laboratory**

WOODS HOLE, MASS.

VOL. XXXIII AUGUST, 1917 NO. 2

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*Vol. XXXIII.* *August, 1917.* *No. 2*

# BIOLOGICAL BULLETIN

## STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

## III. MATURATION AND FERTILIZATION.[1]

J. T. PATTERSON.

### INTRODUCTION.

In the second paper in this series (Patterson and Porter, '17) an account of the spermatogenesis was given. It was shown that males reared from unfertilized eggs carry the haploid number of chromosomes in their germ cells, and that as a consequence the first maturation division is abortive, while the second results in producing two equal spermatids, and hence two similar spermatozoa. The purpose of the present paper is to give an account of the maturation of the egg, and of fertilization.

It was originally planned to report on the maturation process both in fertilized and unfertilized eggs, but my series of the latter has proved to be very incomplete, so that the present account is based entirely on a study of inseminated eggs. However, it is highly probable that maturation is identical in the two types of eggs of *Paracopidosomopsis*, as Silvestri ('06) has found to be the case in *Litomastix*.

It is not a difficult matter to obtain material for the study of maturation. The best way is to place a few non-parasitized moth eggs in a closed vessel containing a large number of female parasites. In the course of an hour each host will have deposited in it several eggs of the parasite—sometimes as many as twenty-five or thirty. The host eggs, thus serving as containers for the parasite eggs, can then be fixed as desired. No difficulty has

[1] Contribution from the Zoölogical Laboratory of the University of Texas. No. 132.

been experienced in cutting the moth eggs into sections from three to five microns thick. Preparations of the entire egg have also been used in the study of maturation. These were made from smears of the host egg.

## The Freshly Deposited Egg.

The freshly laid egg of *Paracopidosomopsis* is a pear-shaped cell, very similar in appearance to that of *Litomastix*, as figured and described by Silvestri ('06). Different eggs vary considerably in size, and to some extent also in shape. In Fig. 2 is shown a typical egg some time after it had been deposited. The average egg measures about 115 $\mu$ in its long axis and 60 $\mu$ at its widest diameter.

The egg may be divided into two parts, the anterior portion or pointed end, and the posterior portion or broad end. At the time of deposition there is no line of demarcation between these two parts, but after the beginning of cleavage they are recognized as the polar region containing the polar nuclei, and the embryonal region consisting of the true embryonic cells.

The egg is surrounded by a thin but tough membrane, probably a true chorion. It is also possible that a very delicate vitelline membrane is present, although this is difficult of demonstration.

The contents of the egg consists of a very finely granular protoplasm in which are found a few scattered yolk or oil spherules (Fig. 8). The fertilized egg contains three distinct bodies, the germinal vesicle, the so-called nucleolus, and the sperm (Fig. 1).

The germinal vesicle is a very conspicuous spherical body, mesuring about 19 $\mu$ in diameter. Its chromatin is in the form of small stellate bodies. There is also present a small but distinct nucleolar-like body (Fig. 1, *n*). Although the position of the nucleus, as well as that of the nucleolus and of the sperm, is very variable, yet it usually lies toward the pointed end of the cell.

The so-called nucleolus in the eggs of polyembryonic insects has given rise to a great deal of discussion as to its origin and fate. Silvestri ('06, '08), who first described and named this body, thought that it arose at an early stage of the oöcyte from the germinal vesicle, but Martin ('14) and Hegner ('14, '15)

have demonstrated that it arises outside the germinal vesicle, and therefore does not represent a true nucleolus. Silvestri ('14) has later admitted the correctness of their conclusions, and proposed the term "oösoma." As to the fate of this body, Silvestri showed that it enters into certain definite cleavage cells, which he believed give rise to germ cells. Hegner has elaborated this idea, bringing it into harmony with his well-known views on germ-line-determinants.

The sperm is invariably found in the posterior half of the egg (Figs. 1, 2, 8), indicating that insemination must take place at any point on the surface of the broad end of the egg. The entire sperm enters the egg (Fig. 8), and soon thereafter forms a small male pronucleus (Figs. 1, 2, 13). There is no evidence that polyspermy ever occurs in the egg of this species, as no egg has ever been found with more than a single sperm in it.

## First Maturation.

As in the eggs of many other Hymenoptera, the maturation divisions of *Paracopidosomopsis* do not result in the formation of distinct polar bodies, but instead only the chromatin undergoes division. Preparation for the first maturation division is seen in the appearance of heterotypic or bivalent chromosomes in the early prophase stage. These are sometimes in the form of V's and crosses, and in the clearest cases eight are present (Fig. 7).

The position of the first maturation spindle is very constant. It lies well toward the anterior end of the cell, with its long axis directed toward the center of the egg (Fig. 8). In the anaphase stage there is present a distinct mid-body at the equator of the interzonal or spindle fibers (Figs. 8, 9). At the close of the division the two groups of chromosomes are not reorganized into nuclei; but lie close together surrounded by clear spaces in the cytoplasm.

In only a few cases have I been able to determine accurately the number of chromosomes that pass to either end of the first maturation spindle. In Fig. 9 the outer, or first polar body group contains eight chromosomes, and at the lower end at least seven can be counted. In Fig. 10 the lower or second oöcyte group

has eight clear chromosomes. In the upper or first polar body group eight are also present, but they are somewhat massed together. These observations indicate that the first maturation division results in separating the two components of each bivalent chromosome, so that the first polar body receives eight whole chromosomes, as does likewise the second oöcyte.

## Second Maturation.

The second maturation occurs soon after the first is completed. The chromatin does not reorganize a nucleus. The first polar body chromatin likewise divides without forming a nucleus.

The second maturation and the division of the polar body may occur simultaneously (Fig. 12), or the polar body division may either precede (Fig. 11) or follow (Fig. 2) the second maturation division. There is therefore no close correlation between the divisions of these two groups of chromosomes.

In Fig. 11 the late anaphase stage of the first polar body division shows eight chromosomes at each end of the spindle. The group of chromosomes lying just below also has eight. This is the second oöcyte group. In Fig. 12 the two division figures are in the late anaphase stage. At the upper end of the polar body spindle (above) six chromatin masses are present, while at the lower end there are eight. The second oöcyte spindle has seven above and eight below. If these two divisions are equational in character, we should expect to find eight chromosomes in each group. Our failure to do so is probably due to the fact that one or more chromosomes in a group may be hidden by some of the other chromosomes.

## Fertilization.

As already stated the egg is inseminated by a single sperm, which lies in the lower half of the newly laid egg. Although the entire sperm enters the egg, only the sperm head becomes transformed into the male pronucleus, the formation of which is completed by the close of maturation.

Of the four groups of chromosomes referred to in connection with Fig. 12, the three lying nearest to the surface of the egg represent the three polar bodies, while the one lying toward the

center is the oötid group. The fate of the polar bodies will be considered later. The oötid group soon forms a small, condensed female pronucleus, which approaches the male pronucleus with which it eventually fuses. The pronuclei come together in the lower half of the egg. Apparently for a considerable period they lie close together before the actual fusion occurs. In Fig. 13 the two condensed pronuclei lie a short distance apart, with a small aster and its tiny centrosome lying between them. This case is interesting because it is the only one in which I have observed either a centrosome or aster in the eggs of this species.

The pronuclei remain apart while they undergo expansion, the female nucleus always being somewhat larger than the male nucleus (Fig. 15). Upon coming in contact with each other, the pronuclei fuse (Fig. 14) to form a single large conjugated, or cleavage nucleus (Fig. 16). Many different stages of conjugating nuclei have been observed in the preparations, one slide alone showing more than a dozen cases.

## Discussion.

These observations show that in the egg of *Paracopidosomopsis* two typical maturation divisions occur, resulting in reducing the sixteen chromosomes of the primary oöcyte to eight in the oötid. It was shown in the second paper of the series that the sperm also receives eight chromosomes. The fertilized egg should therefore have sixteen chromosomes. A study of cleavage divisions shows that this is the case. Fig. 17 is a polar view of a metaphase plate of the first cleavage spindle. It has sixteen chromosomes. In Fig. 18 a side view of an anaphase stage is shown. This is one of the first four blastomeres undergoing division. There are fifteen chromosomes lying toward each end of the spindle, and the sixteenth, just completing its division, lies at the middle of the spindle, on the right.

A study of somatic cells shows that the diploid number of chromosomes is also present in them. Thus the dividing cells of the central nervous system of female larvæ have sixteen chromosomes (Figs. 3, 4). On the other hand, dividing cells from the central nervous system of male larvæ have but eight chromosomes (Figs.

5, 6). This is what would be expected, if males develop parthenogenetically from eggs that have undergone maturation.

AUSTIN, TEXAS,
March 5, 1917.

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## DESCRIPTION OF FIGURES.

### PLATE I.

FIG. 1. Entire egg from a smear preparation, made 23 minutes after oviposition. It shows the germinal vesicle (*n*), the sperm (*s*) and the nucleolus (*no*). ×720.

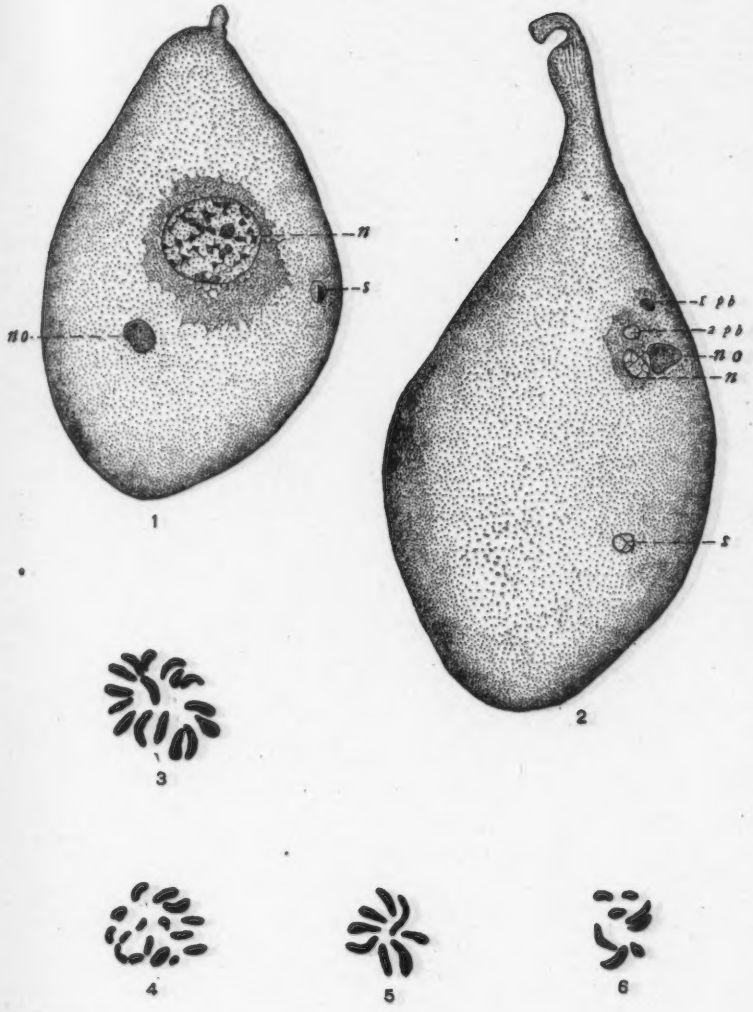
FIG. 2. Entire egg from a smear preparation, showing sperm (*s*), 1st polar body undergoing division (*1 p.b.*), second polar body (*2 p.b.*), egg nucleus (*n*), and nucleolus (*no*). ×737.

FIG. 3. Metaphase plate of dividing cell from central nervous system of female larva. It shows 16 chromosomes. ×3857.

FIG. 4. Same as 3. ×3857.

FIG. 5. Metaphase plate of dividing cell from central nervous system of male larva. It shows 8 chromosomes. ×3857.

FIG. 6. Same as 5. ×3857.




J. T. PATTERSON.

## PLATE II.

FIG. 7. Early prophase of first maturation. The black body close to germinal vesicle is the nucleolus. ×1,240.

FIG. 8. Late anaphase of first maturation. The sperm was drawn in from the next section to the left in the series. ×1,240.

FIG. 9. First maturation spindle. ×1,827.

FIG. 10. First polar body chromosomes above, and chromosomes of the second oöcyte below. ×1,827.

FIG. 11. Anaphase stage of first polar body above, and chromosomes of second oöcyte below. ×1,827.

FIG. 12. Anaphase stages of the first polar body above, and second maturation below. ×1,240.

FIG. 13. Very early stage of the pronuclei, female above, male below. Note tiny aster lying between them. ×1,240.

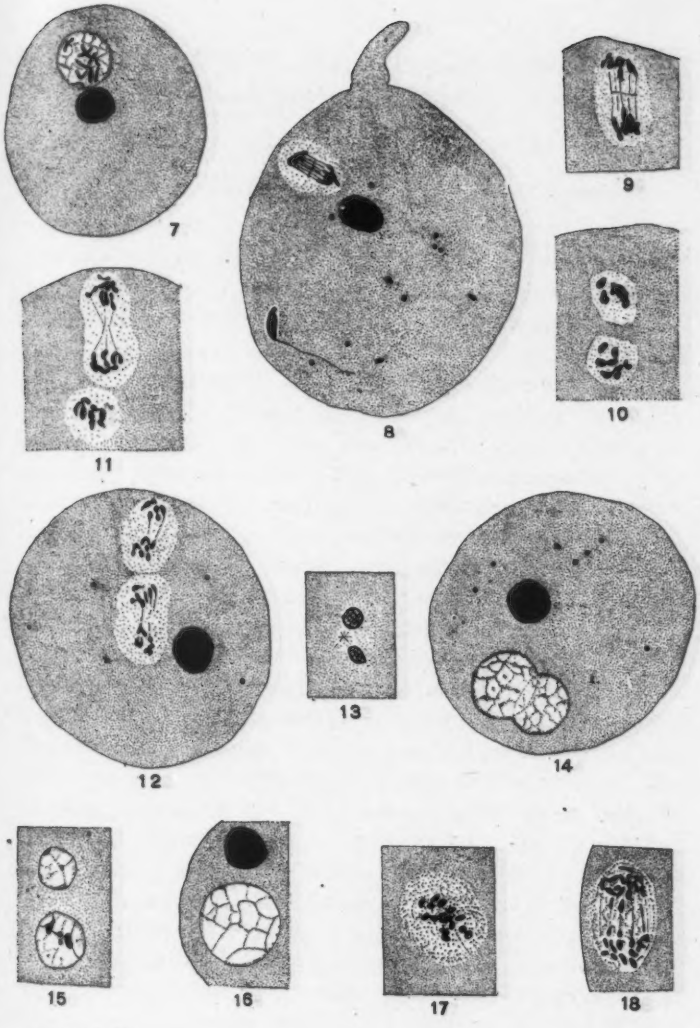
FIG. 14. Fusion of male and female pronuclei. ×1,240.

FIG. 15. Pronuclei, male above, female below. ×1,240.

FIG. 16. Conjugated nucleus. ×1,240.

FIG. 17. Polar view of first cleavage spindle, showing sixteen chromosomes. ×1,827.

FIG. 18. Side view of cleavage spindle of one of the first four cells. There are fifteen chromosomes in each daughter group. The sixteenth chromosome, which has just divided, lies on right side of spindle. ×1,827.




J. T. PATTERSON.

# SOME CHEMOTROPIC AND FEEDING REACTIONS OF PLANARIA MACULATA.

ROSALIND WULZEN.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

When a hungry[1] Planarian is placed in a finger-bowl of water a piece of liver has been lying for some minutes, the worm moves about the dish until it enters the diffusing meat-juice. It pauses at once and extrudes the pharynx. This waves about for a few seconds and is withdrawn. Then the worm takes up its course directly toward the meat and, reaching it, begins feeding. The same reaction is seen when the worm is gliding on the surface film and a bit of egg-yolk is floated near it. This preliminary extrusion of the pharynx, although regularly appearing when hungry worms first come into contact with meat-juice, is very transitory. It does not occur again if the worm leaves the juice and reënters it on its way to the meat or if it is placed in an extract of meat and left to wander about there.

If, however, a section is made through the worm behind the cephalic lobes and anterior to the pharynx the result differs. Directly after the cut the worms move about in an active manner though they eventually come to rest. If while still moving they enter a region of diffusing meat-juice they often stop. The pharynx is extended and remains so; feeding begins upon the juice itself and may continue until the body has swollen and become lighter colored as in ordinary feeding. Often the reaction is not so prolonged but it is always more free and persistent than in uncut worms. From this a natural inference would be that such pieces placed directly upon meat would respond with the feeding reaction, and this is true. The posterior portion of any hungry worm will feed when placed upon a fresh piece of liver.

[1] Worms which had been fasting several weeks were used for these reactions. Well-nourished worms do not furnish good material, as their sensitivity to chemical stimulation is low compared to that of fasting worms.

This result is contrary to that of Pearl,[1] and more extended than that of Bardeen.[2]

A certain degree of independent activity on the part of the pharynx is thus indicated. The same thing could be suspected from the feeding reaction of normal worms. Often a normal worm will come to rest side by side but not in contact with a piece of meat. The pharynx will be extruded and directed toward the meat and will stretch out to reach it to such a degree that the portion of the body to which the pharynx is attached will be pulled out of place, making an indentation on the dorsal surface of the worm. The worm itself will not move at all to help the pharynx, whereas if the pharynx were not extended the chemotropic reaction of the whole worm would carry it directly up to the meat.

There seems to be a twofold chemotropic response, that manifested by the movement of the whole worm toward the meat and that manifested by the movement of the pharynx. Both responses are accurately directed, but when the one is active the other is apparently inhibited. Thus when the stimulation is comparatively weak, the sensitive cells of the skin respond in such a manner that the worm moves directly toward the meat and ordinarily the pharyngeal reflex is entirely in abeyance. When, however, the stimulation is very strong, as upon direct contact with meat or upon the first contact of a very hungry worm with meat-juice, the pharyngeal reflex dominates for a longer or shorter time. When the anterior end of the worm is removed, with it are taken away those portions of the skin which are most sensitive to chemical stimulus. Now a weak stimulus, as that of diffusing juice, calls forth a more pronounced pharyngeal response as though some inhibition were removed.

Some independent chemotropic response seemed so certainly to be located in the pharynx that the question of the activity of the isolated pharynx was raised. If a section is made through the anterior extremity of the pharyngeal sac gentle pressure will force out the pharynx which is now entirely separate from the body. It must be observed that pharynxes so prepared vary as much in sensitivity as do the worms from which they

[1] Pearl, R., *Quarterly Journal of Microscopical Science*, 1902–3, Vol. 46, p. 509.
[2] Bardeen, C. R., *American Journal of Physiology* (1901), Vol. 5, p. 1.

are extracted. But given a sensitive preparation of which any number can be obtained the reaction is as follows: When placed in a dish of water close to a piece of liver, the pharynx elongates actively, its delicate lip is extended into a broad funnel, the walls of which undulate constantly, receiving and being directed by the stimulating substances of the meat-juice. The result is that the funnel is pointed toward the meat, and not only so but the whole pharynx moves forward sometimes several millimeters by a very wormlike series of extensions, contractions and wrigglings until the mouth comes into direct contact with the meat. There is no doubt of the power of the pharynx to orient itself toward the source of diffusing juice and its ability to move toward that source.

The feeding reaction of the pharynx upon reaching the meat is of enough interest to deserve description. Contact of the mouth with the meat causes a wave of peristalsis to pass almost instantly through the pharynx and as a result a mass of food is discharged from the end of the pharynx to which the intestine would normally be attached. Other waves follow in quick succession and the consumed food is discharged at each wave with considerable force. This activity continues for many minutes and results in the accumulation of a surprisingly large heap of food. The shape of the passage through the pharynx is more or less maintained by the masses of food and the little strings of food particles show a tenacity which indicates that the pharynx has added a secretion probably of a mucous nature to the substance passing through it. The experiment can be duplicated with liver extract or yolk of egg dropped carefully on to the bottom of a dish containing water. The pharynx will work its way into the substance and move sometimes a considerable distance in irregular course through it. During the movement it is constantly eating and leaving behind a trail of consumed food. While this reaction is in progress the pharynx bears a remarkable resemblance to a whole organism. The casual observer would immediately call it a living worm. Such entire maintenance of function by an isolated organ is certainly unusual.

# THE ACCESSORY CHROMOSOME IN A FROG POSSESSING MARKED HERMAPHRODITIC TENDENCIES.

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## INTRODUCTION.

In the spring of 1916, the writer, while engaged in determining the effects of starvation upon the development of the germ cells and germ glands of larval frogs, noticed an odd chromosomal body in the germ cells of *Rana pipiens*, which simulated the behavior of an accessory chromosome. Owing to the pressure of other work at that time, the matter was neglected until early in the fall of 1916, when a reëxamination of my material led me to believe that the body I had observed the previous spring was probably an accessory or so-called "sex chromosome."

The present paper is a brief preliminary statement of my observations on the peculiar behavior of this body in the germ cells of *Rana pipiens*.

## LITERATURE.

So far as the writer is aware, there is little cytological evidence of the presence of an accessory, or sex determining factor in the germ cells of amphibia. Miss King in 1912 described dimorphism in the spermatozoa of *Necturus maculosus* resulting from unequal chromosomal division. This brief paper seems to exhaust the findings upon this particular subject.

There is a great mass of literature dealing with cytological studies of the germ cells of Urodeles, but with the exception of Miss King's paper, inequalities in the distribution of chromatin have never been reported.

King, '07, failed to find any indications of accessory chromosomes in the germ cells of *Bufo*. So far as I am aware, no one seems to have worked with *Rana pipiens*, though the material is excellent in every respect.

## Material and Observation.

In the spring of 1916, April 7, to be exact, the writer collected a large number of *Rana pipiens'* eggs from a string of shallow pools near the University campus. The eggs were allowed to develop in the laboratory. The larvæ resulting were used for various experimental purposes, but many were kept until after metamorphosis. Throughout the spring, numbers of the larvæ were killed at odd times up to the period of metamorphosis, in order to obtain a complete series of stages illustrating the normal course of development of the germ glands. It was in the early maturation stages of the germ cells of the older larvæ, that the body simulating the behavior of an accessory chromosome was first observed.

The germ glands of the larvæ were fixed in Flemming's fluid and also potassium-bichromate-acetic; both fixatives gave excellent results. The sections were cut a thickness of 7.5 $\mu$ and stained with iron-alum hæmatoxylin. A counter stain of congo red and orange G was used, but equally good results are obtainable without employing counter stains.

Microscopic examination of the preserved germ glands of the older larvæ revealed the odd fact that the animals were of three kinds: males, females, and larvæ indifferent as regards sex; *i. e.*, hermaphrodites, the germ glands of which contained both male and female cells. Richard Hertwig has fully described sexually indifferent frog larvæ, animals potentially capable of developing into either sex; since his paper first appeared, several other investigators have confirmed Hertwig's findings. In *Rana pipiens* this indifferent condition appears to be fairly common, and is especially marked in some larvæ; the animals retaining their bisexual character, even after metamorphosis, when one or other of the two sexes results.

Further examination of my material showed that in the case of the female larvæ, the maturation processes begin early and usually several weeks before metamorphosis; conversely, in the male, the first maturation changes seldom if ever occur until several weeks after metamorphosis. All stages of the early maturation processes of the germ cells are visible in the glands of female larvæ seven to eight weeks of age.

The oögonial number of chromosomes in the female larvæ appears to be twenty-six. It was possible to make, however, only a few counts, and this number stands, therefore, subject to revision. The male spermatogonial counts will be discussed later. Two oögonial counts were made from a specimen of *Rana catesbiana*, which also gave twenty-six chromosomes as the diploid number. (See Fig. 1.) The size and shape of the chromosomes in the two species of frog appear very similar.

In the post-synizesis stages of the germ cells of the female larvæ of *Rana pipiens*, the chromatin threads which appear from the dense, tangled, heavily staining contraction figure (Figs. 2 and 3), are thick, beaded structures, which have a marked tendency to arrange themselves into the well-known bouquet figures. At this stage all of the chromatin has become arranged in the form of pachytene threads, except a rather large, heavily staining body usually found adhering closely to the nuclear wall (Figs. 4–6). This body is somewhat irregular in shape, though constant in size; the shape usually assumed is that of a triangle, with a minute, thread-like structure at the apex. In the bouquet figures, this chromatin mass orients itself toward one pole of the cell along with the free ends of the pachytene threads (Fig. 6).

Study of a somewhat similar body in the adult male indicates that the body observed in the germ cells of the female larvæ represents probably the accessory chromosomes. At the stage shown in Figs. 4 and 6, the body appears double in most cells. In other cells, for instance Fig. 5, the body is shown as single. Attempts to trace this structure back through the synizesis stage proved futile in my *Rana pipiens* material, owing to certain stains employed by me in connection with a different problem, but in specimens of *Rana catesbiana* larvæ 70 mm. in length, pre-synizesis stages reveal a structure apparently identical with the post-synizesis X-body of *Rana pipiens*.

In female *Rana pipiens*, soon after the synaptic stages of the maturation process occur, the young oöcytes are formed and further study of the *X*-body is impossible in the larval form. Attention was then turned to the germ glands of young male frogs.

Through the kindness of Dr. B. M. Allen, I obtained a number

of sections of the testes of young frogs killed several weeks after metamorphosis had occurred. One of these young frogs was a pseudo-hermaphrodite, over a year old. The testes of this animal contained both ripe spermatozoa and large oöcytes. The germ glands, though containing many oöcytes, were true testes; *i. e.*, the animal was a modified male. All stages of the maturation process were visible; in many follicles the oöcytes and ripe spermatozoa occurred side by side.

The sections had been stained with hæm-alum, counter stained with eosin when received by me. This stain serves very well for the maturation divisions, but is not to be recommended for spermatogonial counts, as it renders the outlines of the chromosomes hazy. As a consequence of this, all of my spermatogonial counts were obtained from sections of testes from other animals, stained with iron-alum hæmatoxylin.

Ten or twelve spermatogonial counts gave twenty-five chromosomes as the diploid number. (See Figs. 7–9.) The chromosomes vary greatly in size and shape, from large V-shaped bodies to small straight rods.

In the hermaphrodite specimen a great many prophases of the first spermatocyte division were found. The chromosomes at this stage were undergoing reduction in number, and appeared as thirteen ring and dumb-bell-shaped bodies of varying size. (See Figs. 10–12.) At slightly later stages, the rings assume the dumb-bell shape also, and are connected end to end by fine linin threads. Still later stages show the linin connectives breaking and the chromosomes becoming scattered through the nucleus. There are thirteen chromosomes visible in the cells. Near the end of the early prophase period, twelve of the typical dumb-bell-shaped prophase chromosomes round off somewhat, becoming oval-shaped; one of the dumb-bell bodies, however, retains its shape; this body, because of its peculiar behavior, I have termed the *X*-body. It is not difficult to identify at this stage because of its large size and dumb-bell shape (Fig. 13).

During the early metaphase stages of the first spermatocyte division, the prophase chromosomes, now oval-shaped, except the *X*-body, line up in the equatorial region of the cell to form the metaphase plate. The *X* chromosome takes its position on

the metaphase plate along with the other chromosomes. The *X*-body is readily detected at this stage by reason of its marked dumb-bell appearance.

Shortly before the chromosomes split, the dumb-bell-shaped *X*-body migrates to one or other pole of the cell, far in advance of the other chromosomes, and comes to lie at the apex of the spindle close to the centrosome (Figs. 14–19). Lateral views of such spindles, with the chromosomes at the metaphase, are difficult to count; the larger chromosomes obscure the smaller in many instances. Usually, however, twelve obviously bivalent chromosomes can be counted at this stage, in the equatorial region of the cell, just previous to splitting of the chromosomes, and one large, dumb-bell-shaped *X*-body, the accessory, at one or the other pole.

The resulting division separates the twelve bivalents into halves, each half migrating toward its respective pole. As a result of this division, an unequal distribution of chromatin to the secondary spermatocytes occurs; one cell receiving twelve chromosomes plus the dumb-bell *X*, the other cell receiving twelve ordinary chromosomes.

A late anaphase of the first spermatocyte division, showing the *X*-body at one pole of the anaphase, the other pole without the accessory, is shown in Fig. 20.

The dumb-bell *X*-body is very conspicuous in many cells in late anaphase stages. It retains the dumb-bell shape, whereas the remainder of the chromosomes are comparatively small, single bodies. In late telophase stages of the first maturation division, all individuality of the chromosomes is lost; the chromosomes clumping together to form a somewhat irregular crescent. In many such stages the accessory chromosome is easily located by the fact that it is so large that half of it projects from the chromatin mass. It may lie horizontal to the long axis of the cell, resting on the other chromosomes, or at times partly imbedded within their mass.

Three first spermatocyte divisions were observed in which the *X*-body appeared as a single and not a dumb-bell-shaped chromosome. Figs. 21–23 show the single nature of the *X* chromosome. In these cells, the *X* is just half the size it appears in other cells.

At the side of the metaphase plate opposite from the *X*-body in one of the three cells, another single body, evidently the product of a precocious division, was observed. This is shown in Fig. 23. In this cell, the dumb-bell accessory seems to have divided, the halves passing to opposite poles. This explanation may also hold for the other two cells in which the *X*-body appears single, though the other half was not observed. There is a possibility that the single appearance of the *X* may be due to end views of the body, though such a possibility seems slight. The very large size of the *X* would, in my opinion, preclude any such possibility.

The secondary spermatocytes, resulting from the division of one of these three cells, the cell in which the *X*-body divided (Fig. 23), would each receive thirteen chromosomes, each cell receiving half of the accessory; whereas, in the case of the remaining two cells, one half the second spermatocytes would receive thirteen chromosomes, the other half, twelve.

Three other cases of dividing first spermatocytes were observed, in which the typical dumb-bell-shaped *X*-body had migrated very early to one pole of the cell, and at the other pole a large, round chromosome appeared.

The two parts of the *X*-body in one cell, were unequal in size, one being considerably smaller than the other. (See Figs. 24–26.) The single, round chromosomes at the opposite pole from the *X*-body in Fig. 24, judging from its size and shape, appears to be the true half of the accessory or *X*.

The behavior of the chromosomes in these three cells is difficult to explain satisfactorily. It is obvious, however, that the secondary spermatocytes resulting from such divisions would receive unequal amounts of chromatin.

Repeated examination of my material convinces me that the normal distribution of chromatin to the secondary spermatocytes, at the first maturation division, is twelve chromosomes, plus the dumb-bell-shaped *X* to one cell, and twelve ordinary chromosomes to the other.

In regard to the case of those cells just described in which the *X*-body appears to have divided precociously, half passing to each pole in advance of the other chromosomes, it is probable

that we are dealing with abnormal divisions. The same is true, of course, of those cells in which the accessory either divided precociously, half passing to each pole, but one half becoming linked with a smaller chromosome or perhaps a chromatoid body (see Fig. 24), or else the halves of the *X* are unequal.

However this may be, it should not be forgotten that the animal in whose germ cells these anomalies of chromosomal distribution occur, is bisexual; *i. e.*, with the germinal products of both sexes in its glands. There may, or may not be, a connection between such abnormalities of chromatin distribution at the maturation divisions, resulting, presumably, in the production of three kinds of spermatozoa, and the fact that in certain strains of this species of frog, males, females and animals possessing marked hermaphroditic tendencies occur.

It is obvious, however, that the presence of oöcytes in my specimen is not due to any inequality in chromatin sharing of the daughter cells at the maturation divisions, because the oöcytes are formed long before the male maturation period occurs. Such oöcytes may be due to unequal chromosomal division at an earlier period; perhaps in the spermatogonia or even primordial germ cells. In this connection it may be mentioned that in those follicles containing oöcytes, and they are far from uncommon in my specimen, spermatids and mature spermatozoa are also usually found. The two kinds of cells may be found side by side. The fact that the germinal products of both sexes are usually found together in the same follicle suggests that perhaps both oöcytes and spermatozoa may be products of the same cell originally. It might not be amiss to point out here that the oöcytes and spermatozoa found in the same follicles of this animal represent advanced stages in the germ cell cycle, not found in the surrounding follicles. The surrounding follicles contain spermatogonia and prophases of first maturation divisions, stages much younger than spermatozoa and egg formation.

### The Second Spermatocytes.

The second spermatocytes resulting from the first maturation division divide at once without an observable period of rest. The telophase of the first spermatocyte division leaves the chro-

matin matter a solid, somewhat irregular crescentic mass. This mass soon rounds off its angularities and arranges itself along the equatorial region of the cell. The outlines of the individual chromosomes are, for the most part, obliterated at this stage. No further reduction of chromosomes occurs at this division. The dumb-bell-shaped *X*-body is not difficult to identify in the second spermatocyte at the metaphase. Its identification is rendered easy by its large size and peculiar dumb-bell shape. (See Figs. 27–28.) Part of the dumb-bell usually is seen projecting above the metaphase plate. This is well shown in Figs. 27–28.

All attempts to determine the chromosomal number in secondary spermatocytes in my bisexual specimen proved futile, owing to the hæm-alum stain. The dumb-bell *X*-body divides along with the rest of the second spermatocyte chromosomes. No attempt was made to trace the further history of the accessory body in the spermatids and spermatozoa. I hope to take up this matter in another paper.

## The Chromatoid Body.

A number of investigators of amphibian spermatogenesis have observed and reported the presence of an oval, homogeneous chromatoid body in the cytoplasm of the germ cells.

King, '07, reported it in *Bufo*, and claims it forms the acrosome of the spermatozoon. Herman has found a similar body in the spermatids of *Salamandra*. Recently Bachhuber reported the presence of such a body in the spermatocytes of the rabbit. It has also been reported for other animals.

In *Rana pipiens* the chromatoid body is readily found in the cytoplasm of the secondary spermatogonia. I have never observed it in the primordial germ cells of very young larvæ. This structure is of fairly large size, oval in shape and stains readily. It bears considerable resemblance to an extruded nucleolus, though probably has an extra-nuclear origin. The position of the body in the cytoplasm varies considerably. In some cells it adheres closely to the nuclear wall; in other cells it is found near the periphery of the cell. During spermatogonial division it does not appear to divide, though it occurs among the

spindle fibres; actual division of the body was observed but once. (See Fig. 29.) The body is readily distinguished from the chromosomes by its peculiar character, and the position it takes during cell division.

In the primary spermatocytes the chromatoid body is conspicuous in the cytoplasm. Whether it does or does not divide at the first maturation division was not ascertained. In Fig. 24 it is shown passing to one pole, apparently undivided.

I make mention of the behavior of this structure here, in order to disarm, beforehand, the criticism which perchance might arise in connection with this paper, that I may have mistaken the chromatoid body for an accessory chromosome. The origin and fate of this peculiar body in *Rana pipiens* seem worthy of further investigation.

## Summary and Conclusion.

1. The oögonial number of chromosomes in *Rana pipiens* and *catesbiana* appears to be twenty-six.

2. The spermatogonial number is twenty-five in *Rana pipiens*.

3. In the synaptene stage of the germ cells of the female *Rana pipiens* larvæ, a chromatin body is found which simulates the behavior of an accessory chromosome.

4. The reduced number of chromosomes in the male of *Rana pipiens* is thirteen.

5. At the first maturation division of the germ cells of a frog possessing marked hermaphroditic tendencies, there is an unequal distribution of chromatin to the daughter cells, the inequality varying in different cells.

6. Perhaps this unequal distribution of chromosomes at the spermatocyte division and the resulting inequality of chromosomal distribution to the spermatozoa may account for the fact that males, females, and animals possessing marked hermaphroditic tendencies are found in this species of frog.

In conclusion, I wish to acknowledge my indebtedness to Dr. B. M. Allen for the loan of several valuable slides and to Dr. W. R. B. Robertson for several excellent suggestions.

April 11, 1917.

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## EXPLANATION OF FIGURES ON PLATES I–VI.

All figures were drawn with the aid of a camera lucida. Spencer 2 mm. apochromat oil immersion objective and oc. 18 used. Drawings are accurate with reference to the nuclear material and chromatoid body. The cytoplasm, however, is represented in the conventional way. *ch*, chromatoid body; *C*, centrosome.

### PLATE I.

FIG. 1. Polar view of metaphase plate in dividing oogonia from ovary of *Rana catesbiana*, twenty-six chromosomes are present.

FIGS. 2 and 3. Synizesis stages in young oöcytes of *Rana pipiens* larvæ.

FIGS. 4, 5 and 6. Young oöcytes of *Rana pipiens*, showing the pachytene threads and the X-chromosome adhering to the nuclear wall.

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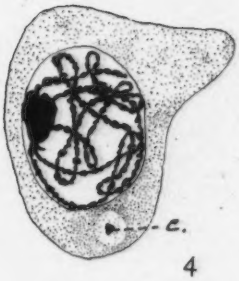


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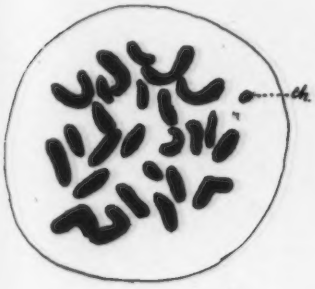
PLATE II.

FIGS. 7, 8 and 9. Spermatogonia of *Rana pipiens*, showing twenty-five chromosomes.

FIGS. 10, 11, 12 and 13. Spermatocytes, showing prophases of the first maturation division.

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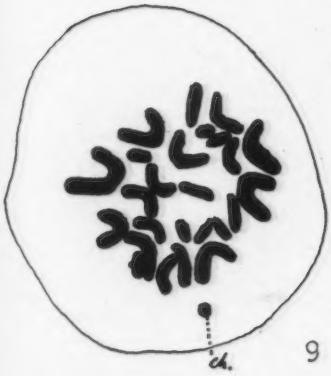


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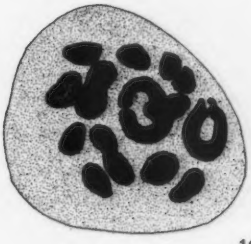

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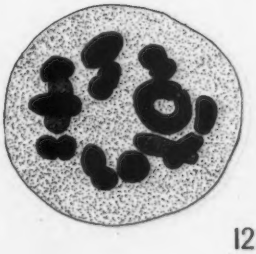

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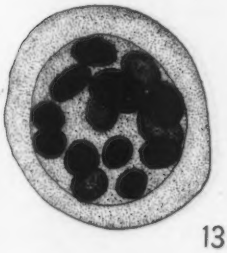
PLATE III.

FIGS. 14, 15, 16, 17, 18 and 19. Spermatocytes, showing first maturation division and the migration of the accessory chromosomes to one pole.



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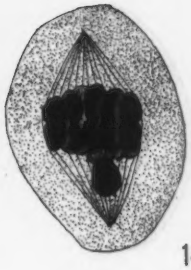

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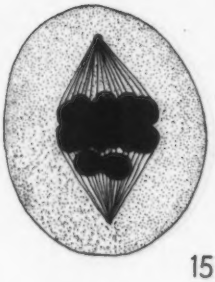

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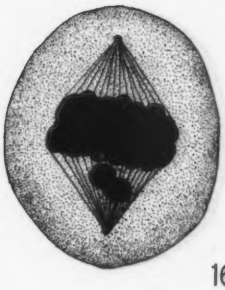

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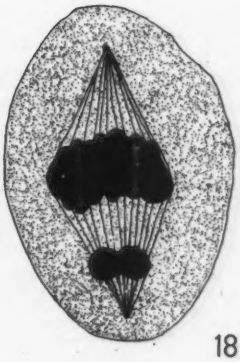

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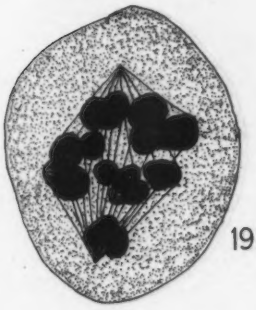
PLATE IV.

FIG. 20. Late anaphase of a dividing first spermatocyte showing the accessory chromosome at one pole.

FIGS. 21, 22 and 23. First maturation divisions showing single nature of accessory chromosome.

FIG. 24. First maturation division showing accessory chromosome at one pole and single round chromosome at opposite pole.



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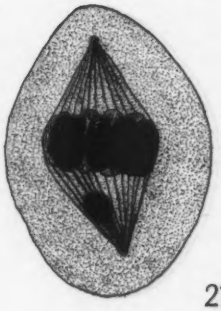

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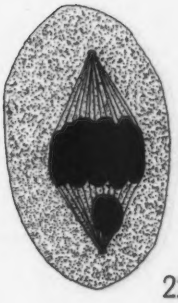

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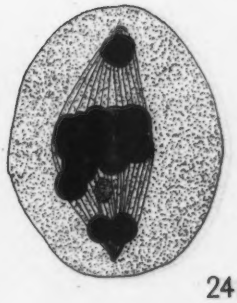

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PLATE V.

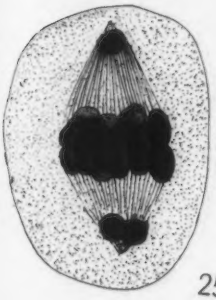
FIGS. 25 and 26. First maturation divisions showing accessory chromosome at one pole of the cell; single chromosome at opposite pole.

FIGS. 27 and 28. Second spermatocytes showing the large accessory chromosome.

FIG. 29. Anaphase of dividing spermatogonium showing division of the chromatoid body.

FIG. 30. Spermatocyte of *Rana catesbiana* with two chromatoid bodies in the cytoplasm.

FIG. 31. Spermatogonium of *Rana pipiens* showing large chromatoid body.



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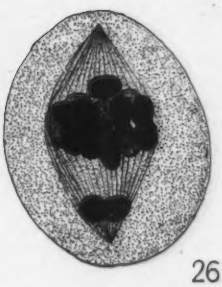

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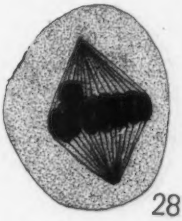

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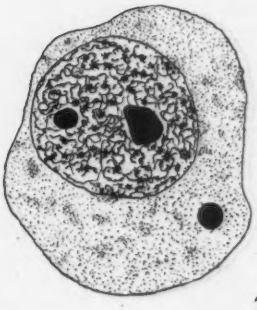

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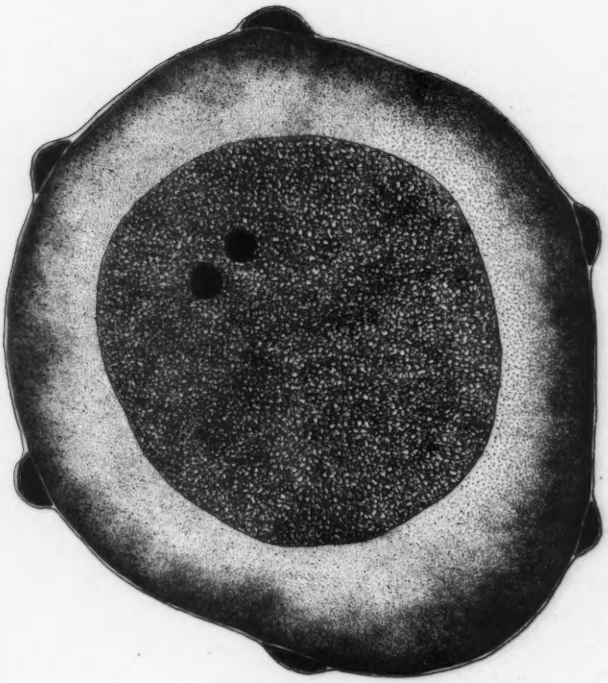
PLATE VI.

FIG. 32. Oöcyte from the testis of young frog



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# THE EXPERIMENTAL PRODUCTION OF HYPOTYPICAL OVARIES THROUGH UNDERFEEDING. A CONTRIBUTION TO THE ANALYSIS OF STERILITY.[1]

LEO LOEB.

In former communications we described a condition of the ovaries in which the follicles become atretic, usually before they have reached medium size. Mature follicles are not produced under these conditions and sterility ensues which persists as long as this condition lasts.[1] Such ovaries we designated as hypotypical. We observed those changes especially in guinea pigs in which the corpora lutea had been burnt at a certain period after ovulation. We must assume that the burning of a part of the ovary caused a "tissue shock" in the remaining part of the ovary which, without killing the follicles, weakened the granulosa cells and thus prevented their further development and caused their early disintegration. In addition we found this change in a number of other animals, some of which had not yet been in heat, and had consistently refused copulation, although their age was such that we might have expected to find them sexually active. In some other cases in which as the microscopic examination showed, ovulation had failed to take place after the conclusion of the last sexual period notwithstanding the degeneration of the corpora lutea, hypotypical ovaries were likewise found. This suggests that this condition may be the cause of long-lasting or perhaps perpetual sterility. This hypotypical condition of the ovaries is of theoretical interest, inasmuch as it represents a state of low developmental energy of an organ that is normally in a condition of constant change. From a practical point of view it is of importance because it can lead to sterility of the gonads. It seemed to us therefore of interest

[1] From the Department of Comparative Pathology, Washington University Medical School, St. Louis.

[2] Leo Loeb, *Zentralblatt f. Physiologie*, 1911, XXV., No. 9; *Virchow's Archiv*, 1911, CCVI., 278.

to carry out further experiments in order to throw additional light on the causes of the hypotypical condition of the ovaries. It occurred to us that a common cause might underlie all those cases in which a local injury of the ovaries was not present, and that lack of proper nourishment might be an important factor in this form of temporary sterility. We therefore carried out four series of experiments in which female guinea pigs received the same kind of food as usual, viz., oats and a little grass or green vegetables, but both in much reduced quantity. We shall discuss our experiments in the inverse order in which they were done. In all cases but one both ovaries were cut in serial sections after the completion of the experiment. In one animal only one ovary was cut serially.

## IV. Series.

Five guinea-pigs were used in this experiment. (See Table I.)

Table I.

IV. Series.

| Guinea Pig. | Original Weight. | Loss of Weight in Per Cent. of Original Weight. | Period During Which Loss of Weight Occurred. | Result. |
|---|---|---|---|---|
| *A*.... | 294 grams | 28% | 12 days | Animal weak at end of experiment; about 10–13 days previously ovulation. Hypotypical ovaries of first order. |
| *B*..... | 309 grams | 21% (in 5 days) | 6 days | Found dead. Good-sized follicles in granulosa-Degeneration, preparatory to the development of hypotypical ovaries. No ovulation. |
| $B_1$.... | 288 grams | 27% | 10 days | Weak, killed. Hypotypical ovaries of second order. Delayed ovulation. |
| *C*..... | 331 grams | 36% | 14 days | Weak, killed. Hypotypical ovaries of second order. Delayed ovulation. |
| *D*..... | 342 grams | 34% | 14 days | Weak, killed. Necroses in liver. Hypotypical ovaries of first order, delayed ovulation. |

### *Microscopic Examination.*

*Guinea Pig A.*—Had lost 28 per cent. of its weight twelve days after beginning of feeding experiment.

*In the ovaries:* small and small to medium follicles. In the

latter slow disintegration of granulosa cells takes place and connective tissue grows in. Small follicles in various stages of connective tissue atresia; many follicles in the last stage of atresia, with relative prominence of the theca interna. Remnants of old retrogressing corpora lutea: yellowish stained vacuolar cells surrounded by dense hyaline fibrous tissue, traversed by thick-walled blood vessels. In addition we find well-preserved small corpora lutea with capillaries and myxoid connective tissue in center. Mitoses are not present in the corpora lutea. The *uterus* thin, with low cylindrical surface epithelium and small glands. The mucosa is fibrillar. No sign of a cell-layer.

We must assume that ovulation had taken place at least as early as ten days before examination or even somewhat earlier. The structure of the corpus luteum and the absence of all proliferative changes in the uterine mucosa suggest this conclusion. The new corpora lutea remain small as a result of the insufficient food. The follicles develop only to small-medium size and then undergo atresia. Thus hypotypical ovaries are produced. The uterus is thin, atrophic.

*Guinea Pig B.*—Examined six days after beginning of feeding experiment; had lost approximately 25 per cent. to 27 per cent. of its weight. This animal was found dead; examination several hours after death.

*In the ovaries* good-sized follicles with granulosa degeneration; good small and small-medium follicles; various stages of connective tissue atresia. Neither a preserved nor a retrogressing corpus luteum present. The *uterus* is thin with the usual low cylindrical epithelium of surface and small glands. Some epithelial cells at top of papillæ show mucoid transformation; fibrillar mucosa. In this case we have to deal with ovaries which are not yet hypotypical, but which apparently undergo some changes tending towards a hypotypical condition; the larger follicles degenerate and the smaller follicles would in all probability fail to develop to their full size. In this animal ovulation had failed to take place throughout a considerable period of time.

*Guinea Pig* $B_1$.—Examined ten days after beginning of feeding experiment; had lost 27 per cent. of its weight. *Ovaries* show

small and small-medium good follicles. In the latter follicles a slow solution of granulosa sets in. We see some follicles of almost medium size without granulosa, but as yet without connective tissue ingrowth. Follicles in various stages of connective tissue atresia, especially numerous follicles in the last stages of connective tissue atresia with relative prominence of the theca interna. A small yellow fibrous body, the remnant of an atretic corpus luteum is present.

*Uterus* thin with the usual relatively low epithelium and small glands and fibrillar mucosa. In this case after degeneration of the corpus luteum of the former period, a new ovulation failed to take place and the follicles did not grow to normal size. A hypotypical ovary resulted.

*Guinea Pig C.*—Examined fourteen days after beginning of feeding experiment; had lost 36 per cent. of its weight. *Ovaries* small, living corpora lutea with small center of fibrous tissue. There is perhaps one mitosis in a Lutein cell. In addition there are fibrous, yellow bodies, the remnants of degenerated corpora lutea. Small and small-medium good follicles. Medium follicles show slow granulosa degeneration. The ingrowth of connective tissue into the follicles is probably somewhat delayed; there are a number of follicles with loss of granulosa and as yet without ingrowth of connective tissue. Various stages of connective tissue atresia of follicles are seen. Atretic follicles in the last stage of atresia and with a large mantle of theca interna are frequent. *Uterus* shows the same character as in the other animals; relatively low epithelium, small glands, fibrillar mucosa. Mitoses nowhere visible.

We may assume that in this case the last ovulation took place very soon after the beginning of the feeding experiment. No trace of proliferation was visible in the uterine mucosa. This indicates that at least ten days had elapsed since the last ovulation, a conclusion in accordance with the character of the corpora lutea. The follicles instead of growing to normal size after ovulation, remained small and underwent premature atresia.

*Guinea Pig D.*—Examined fourteen days after beginning of feeding experiment; had lost 34 per cent. of its weight. *Ovaries* with small and small-medium good follicles. In the latter slow

granulosa degeneration sets in, leading to a complete solution of the granulosa cells with subsequent ingrowth of connective tissue resulting in various stages of connective tissue atresia. Follicles in the last stages of connective tissue atresia form a prominent part of the ovaries. Neither a new corpus luteum nor a remnant of a retrogressing corpus luteum visible. The last ovulation had therefore taken place more than four weeks previous to examination. The *uterus* is thin, with relatively low epithelium and small glands. Much secretion is present in surface epithelium and some gland ducts. The mucosa is thin and fibrillar.

In the four guinea pigs in which ten days or more had elapsed since the beginning of the experiment the ovaries had become hypotypical; while in guinea pig B, which was examined six days after the beginning of the experiment, degenerative changes took place in the large follicles, probably preparatory to the ensuing hypotypical changes. In this animal the relative loss in weight had been almost as marked in six days as in the other guinea pigs in a longer period. It seems therefore that a minimum time has to elapse before the hypotypical condition of the ovaries is established. The latter can be obtained in one of the following two ways: First, after the degeneration of all the good-sized follicles following ovulation, the small follicles fail to reach full size. Before they have grown to medium size, the granulosa cells perish and connective tissue grows in. Second, the large follicles degenerate perhaps at a somewhat accelerated rate without an ovulation having preceded this change, and the small follicles fail to reach medium size. The granulosa of the small-medium follicles does not show the karyorrhexis affecting simultaneously a large number of the granulosa cells and characteristic of the beginning atresia of large follicles; isolated granulosa cells may, however, show karyorrhexis.

In all of the animals with exception of guinea pig *D* ovulation had probably taken place within the last month; and in two animals within the last two weeks preceding the time of examination. We are therefore justified in assuming that at the beginning of the experiment the ovaries had been fully developed in these animals in accordance with their initial weight and age. The lack of a sufficient amount of food however prevented

apparently the corpora lutea from reaching full size. It is furthermore probable that toward the end of the experiment the ingrowth of connective tissue into the cavity of the atretic follicles was somewhat delayed. Still the difference in the behavior of granulosa cells and connective tissue is striking. While the former becomes dissolved as a result of the underfeeding, the latter is still active and organizes the cavity of the follicle. The uterus in all cases was thin, and its mucosa did not show any sign of growth. The ova represent a further element still active under these conditions. In the small-medium follicles in process of atresia mitotic and amitotic divisions of the nuclei take place, as well as a division of the superficial part of the egg into cell-like fragments in which however usually nuclei are not visible.

III. SERIES. (See Table II.)

Fifteen guinea pigs were used in this series, five, weighing between 534 and 380 grams at the beginning of the experiment, had been bred in Missouri; ten younger ones, varying in weight between 378 and 259 grams, were obtained from Iowa. Three of these animals were well fed during the period of the experiment and served as controls. In addition two other control guinea pigs were examined at the beginning of the experiment. We shall first describe ovaries and uterus of the control animals.

1. *Control Animals.*

*Guinea Pig No. 3.*—Weight Oct. 18, 474 grams. Nov. 11, twenty-four days after beginning of experiment, weight 613 grams. The animal was found pregnant. The ovaries were normal; they contained good follicles of all sizes, large follicles in granulosa degeneration, good mature follicle as well as mature follicles with ganulosa degeneration. Follicles in various stages of connective tissue atresia. Good corpora lutea as well as degenerated corpora lutea (yellow-fibrous bodies). The *mammary gland* was typically proliferating.

*Guinea Pig No. 9.*—Weight Oct. 18, 286 grams; weight Nov. 2, 375 grams; examined Nov. 4. *Ovaries:* Good corpora lutea, the cavity of which was filled with connective tissue, retrogressing corpora lutea; good small and medium follicles, various stages

of connective tissue atresia, especially the later stages are well represented. The *uterus* shows a predeciduomatous cell layer with mitoses in the mucosa. The gland fundi with high cylindrical cells. The *mammary gland* consists of acini with relatively high, well-staining epithelium, but without mitoses, and with rather cellular connective tissue between the acini. We have here to deal with normal sexual organs in an animal at a period about five days after ovulation.

*Guinea Pig No. 12.*—Weight Oct. 18, 263 grams. Weight Nov. 11, 352 grams. In the left horn of the uterus two pregnancies were found. *Ovaries:* Good corpora lutea; their central cavities are filled with connective tissue; good follicles of all sizes. Follicles with granulosa degeneration; good mature follicles; various stages of connective tissue atresia. *Uterus* is that of pregnancy. In addition to these three guinea pigs, two other guinea pigs obtained from Iowa (*1a* and *1b*) were examined as controls at the beginning of the experiment.

*Guinea Pig No. 1a.*—Weight Oct. 19, 378 grams. *Ovaries:* Good corpora lutea; the central cavity is almost completely filled by connective tissue, but a small remnant is still left unorganized. Good small and medium and almost large follicles. Some follicles in the last stages of connective tissue atresia. *Uterus* well developed with a predeciduomatous cell layer in mucosa. Fundi of glands with high cylindrical cells. *Mammary gland:* Acini with good cuboidal epithelium and a few mitoses; cellular stroma. We have here to deal with normal sexual organs in an animal about five to six days after ovulation.

*Guinea Pig No. 1b.*—Weight Oct. 19, 271 grams. *Ovaries:* Good follicles of all sizes, including large and mature follicles. Much hyperemia around the mature follicles, capillaries in theca interna of these follicles much enlarged. Some follicles in various stages of connective tissue atresia, but relatively few follicles in the last stage of connective tissue atresia. No sign of new or degenerating corpora lutea. The *uterus* showed high cylindrical surface epithelium; gland ducts with high epithelium; gland fundi with lower epithelium. The mucosa is hyperemic and succulent and papillomatous. A few mitoses in surface epihelium and at the opening of the glands. *Mammary gland*

very small. A few acini with good cuboidal-cylindrical epithelium with a few mitoses; stroma between acini cellular. The animal was apparently for the first time in her life in the period of heat. We see then that all the control guinea pigs at the beginning and at the end of the experiment were perfectly normal; they were sexually mature and underwent the typical sexual cycle.

### 2. *Undernourished Animals.*

In order to shorten the description of our findings, we shall from now on designate ovaries in which atresia sets in when the follicles are as yet of small or small to medium size as hypotypical follicles of the first order, and ovaries in which atresia sets in at a slightly more advanced stage of the development of the ovaries, in follicles which have reached or almost reached medium size as hypotypical ovaries of the second order. The uterus is in those cases thin and shows the characteristics described in the fourth series. We shall designate such a uterus as hypotypical.

*Guinea Pig No. 1.*—Still active at time of examination. Abortion had taken place recently. Ovaries showed corpora lutea with marked vacuolization and thick-walled vessels. The vacuolization affects all parts of the corpus luteum, but especially the periphery. These are the corpora lutea of the preceding pregnancy; following abortion they underwent degenerative changes. Atretic yellow bodies (the remnants of degenerated corpora lutea); good small and medium follicles and only an exceptional good large follicle. The large majority of the large follicles showed granulosa degeneration; follicles in various stages of connective tissue atresia. One almost mature follicle. *Uterus:* Cells of surface epithelium high and large with prominent cytoplasm; glands with rather high epithelium, but with less prominent cytoplasm; many mitoses in surface epithelium; fibrillar mucosa. At one place there are signs that regeneration had taken place and that a decidua had been present. *Mammary Gland:* Large. Acini with good cuboidal epithelium and small lumen. Some colloid in lumen. Some large vacuoles in epithelial cells. No mitoses. Cellular stroma. Some mononu-

clears and polynuclears in stroma. Beginning secretion following abortion. In this case the underfeeding was probably responsible for the abortion. In the controls otherwise held under the same conditions, but given sufficient food, pregnancy proceeded normally. Ovaries, uterus and mammary gland corresponded to the stage of the sexual cycle of this animal.

*Guinea Pig No. 2.*—Weight Oct. 18, 464 grams; Nov. 18, 302 grams. Following this date it received a full ration of food for a few days and on Nov. 25 it had regained a part of its loss, weighing now 357 grams. Underfeeding was again resumed. On Nov. 30, it weighed 303 grams and it was examined. *Ovaries:* Two young corpora lutea with dilated capillaries. There is in the corpora lutea a central cavity surrounded by a little peripheral connective tissue. Mitoses are found in the capillaries and in the central connective tissue. Atretic yellow bodies with vacuoles and thick vessels are also present. Small and small-medium good follicles with a very thin layer of granulosa which even around the egg is imperfect. Some mitoses in the granulosa cells. In other small-medium follicles granulosa is lost. Follicles in various stages of connective tissue atresia. The *uterus* shows a high cylindrical surface epithelium, glands with cuboidal cells and a very cellular myxoid mucosa. The *mammary gland* consists of good acini without mitoses; it presents an intermediate character. Ovulation had taken place about 2–3½ days previous to the examination. It is probable that following ovulation the ovaries ceased to develop normally and are becoming hypotypical. The solution of granulosa cells in small-medium follicles suggests such an interpretation. We may assume that probably following the transitory gain in weight a few days before examination ovulation occurred. For a considerable period previous to the last ovulation, ovulation had failed to take place, probably in consequence of the underfeeding. We find therefore only remnants of degenerated corpora lutea, representing the corpora lutea of the preceding cycle. The underfeeding did not prevent mitotic division in various structures, nor did it prevent the development of a slightly predeciduomatous condition of the uterine mucosa.

*Guinea Pig No. 4.*—The animal was in a dying condition,

when examined. Only one *ovary* was examined microscopically. Small and small-medium good follicles; some granulosa degeneration in other small-medium follicles. Various stages of connective tissue atresia; especially many follicles in the last stages of connective tissue atresia, much interstitial gland. No corpus luteum present. *Uterus* showed low cuboidal-cylindrical epithelium of surface and glands without mitoses; fibrillar mucosa. The *mammary gland* consisted of dense fibrous tissue with a few ducts and acini; acini with cuboidal epithelium and without colloid in the lumen. At places the stroma was somewhat more cellular. In this case we can be sure that the ovaries were hypotypical, although only one ovary had been examined. The condition of the uterus makes it certain that a recent ovulation had not taken place. The animal has been markedly affected by the underfeeding, it was in a dying condition at the end of the experiment.

*Guinea Pig No. 5.*—The animal was found dead. Hypotypical ovaries of the second order. Some medium follicles with granulosa degeneration. Two retrogressing small vacuolar corpora lutea with thick vessels. *Uterus* is hypotypical. In this case ovulation had been delayed.

*Guinea Pig No. 6.*—Examined in a dying condition. Hypotypical ovaries of the second order. Some almost medium follicles with a thin wall of granulosa. In follicles of almost medium size some granulosa degeneration. No corpus luteum or remnant of a corpus luteum visible. *Uterus* hypotypical.

*Guinea Pig No. 7.*—Found dead. No noticeable postmortem changes. Hypotypical ovaries of the first order. Small degenerating corpora lutea with vacuolar cells and thick vessels. *Uterus* hypotypical. In this case a new ovulation had not yet taken place, although the corpus luteum of the preceding period was degenerating. The loss of weight in this case had been rapid.

*Guinea Pig No. 8.*—Found dying. Hypotypical ovaries of the second order. There is present a retrogressing small corpus luteum with vacuolar cells. Uterus hypotypical. Mammary gland shows dense fibrous tissue and small gland ducts without mitoses. Ovulation was delayed.

*Guinea Pig No. 10.*—Found dead, slight postmortem changes.

## TABLE II.

### III. SERIES.

| Guinea Pig. | Original Weight. | Loss of Weight in Per Cent. of Original Weight. | Period During Which Loss of Weight Occured. | Result. |
|---|---|---|---|---|
| No. 1.. | 534 grams | 28% | 14 days 30% in 24 days | Abortion. Typical cycle, delayed ovulation. |
| No. 2.. | 464 grams | 23% | 33 days 35% in 43 days | Period of refeeding preceded examination. Ovulation with growth in uterine mucosa a few days previous to examination. Ovulation had been suspended for a long period of time. Beginning hypotypical ovaries. |
| No. 4.. | 415 grams | 31% | 14 days | Dying. Hypotypical ovaries of first order. |
| No. 5.. | 380 grams | 28% | 12 days. | Found dead. Hypotypical ovaries of second order. Delayed ovulation. |
| No. 6.. | 313 grams | 27% | 14 days | Dying. Hypotypical ovaries of second order. |
| No. 7.. | 302 grams | In 5 days about 9% of the weight lost; had lost some weight in the preceding 10 days | 7 days | Hypotypical ovaries of first order. Delayed ovulation. |
| No. 8.. | 305 grams | 27% | 7 days | Dying. Hypotypical ovaries of second order. Delayed ovulation. |
| No. 10. | 292 grams | In 5 days lost 12% of its weight. Died 2 days later | 7 days | Found dead. Hypotypical ovaries of first order. Delayed ovulation. |
| No. 11. | 283 grams | 26% | 7 days | Dying. Hypotypical ovaries of second order. Delayed ovulation. |
| No. 13. | 259 grams | Lost in 5 days 13% of its weight | 7 days | Hypotypical ovaries of second order. Delayed ovulation. |
| | | *Controls.* | | |
| No. 3.. | 474 grams | 29% | 24 days | Well-developed pregnancy. Normal ovaries. |
| No. 9.. | 286 grams | 31% | 15 days | 5 days after ovulation. Ovulation evidently occurred at normal intervals. |
| No. 12. | 263 grams | 34% | 24 days | Pregnancy. Normal ovaries. |
| | | *Additional Controls; Examined at Beginning of Experiment.* | | |
| No. 1a. | 370 grams | | | About 5–6 days after ovulation. Normal ovaries and uterus. |
| No. 1b. | 271 grams | | | Animal in first heat. Normal sexual organs. |

Hypotypical ovaries of the first order. A small vacuolar corpus luteum. Hypotypical uterus. In this case again delayed ovulation.

*Guinea Pig No. 11.*—Was found dying. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Uterus hypotypical. Delayed ovulation.

*Guinea Pig No. 13.*—Found dead. Some postmortem changes. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Hypotypical uterus.

In this series all the guinea pigs show hypotypical ovaries after a loss of weight of approximately 26–31 per cent. in the course of one to two weeks. In Guinea Pig No. 13 we find in a period of five days a loss of 13 per cent., but in the last two days before death a further considerable loss of weight had in all probability occurred. In 2 cases (Guinea Pigs Nos. 1 and 2) we do not yet find hypotypical ovaries. In the first animal, however, abortion took place toward the end of the experiment. We may attribute the abortion to the lack of a sufficient quantity of food. Abortion is not usual among guinea pigs without a preceding experimental interference. Following the abortion a new ovulation had not yet taken place at the time of examination. In the second animal (Guinea Pig No. 2) ovulation had taken place shortly before the conclusion of the experiment, but there were indications of a beginning hypotypical condition of the ovaries following the ovulation. In these two cases we have in the first place to deal with somewhat heavier animals, and secondly, a slightly smaller relative loss of weight was distributed over a longer period of time. It is probable that the greater initial weight of these guinea pigs caused a greater resistance to the underfeeding; their general condition was less affected by the underfeeding and consequently we find as yet no marked changes in ovaries and uterus. In the five control animals of this series the sexual cycle took its normal course; pregnancy as well as ovulation proceeded normally.

### II. Series. (See Table III.)

Five guinea pigs were used in this experiment. Guinea Pig No. 1 served as control, the other four were undernourished and

received only water and a few leaves of grass each day. The experiment began June 30, 1916. For briefness sake, only abstracts of the descriptions can be given.

TABLE III.

II. SERIES.

| Guinea Pig. | Original Weight. | Gain or Loss in Weight in Per Cent. of Original Weight. | Duration of Experiment. | Result. |
|---|---|---|---|---|
| No. I, control. | 438 grams | A loss of 15 grams | 9 days | At end of pregnancy. Normal ovaries and uterus. |
| No. II. | 462 grams | −29% | 9 days | Perhaps first change in direction toward development of hypotypical ovaries. Probably abortion a short time previously. Delayed ovulation. |
| No. III. | 454 grams | −38% | 9 days | Not hypotypical ovaries, but beginning change tending in that direction. Delayed ovulation. |
| No. IV. | 428 grams | −35% (in 9 days) | 10 days | Not yet hypotypical follicles but probably some retardation in the development of follicles. Delayed ovulation. Animal very weak at end of experiment. |
| No. V. | 439 grams | −34% (in 9 days). Then a gain of 24% of the original weight and of 31% of the lowest weight reached July 9 | 21 days. (9 days deficient and 11 days full feeding) | No mature follicles and delayed ovulation; ovaries otherwise normal. |

*Guinea Pig No. 1.*—Control. In one horn of uterus one embryo: near end of pregnancy. In this case the condition of *ovaries*, *uterus* and *mammary gland* corresponded to the last stage of pregnancy. In the ovaries large and mature follicles were present. The mammary gland was proliferating.

*Guinea Pig No. II.—Ovaries:* Good small and medium follicles, some larger follicles in granulosa degeneration and perhaps one well preserved large follicle. Most follicles seem to degenerate, when in medium size. Various stages of connective tissue atresia. Large vacuolar corpora lutea with thick vessels. Atretic yellow body (remnant of corpus luteum). *Uterus:* Relatively low cuboidal-cylindrical epithelium of surface and glands. Some

mitoses in surface epithelium, nowhere else; fibrillar mucosa. *Mammary gland:* Large gland, with acini of various sizes, often filled with colloid material. Fibrous or fibrillar stroma, with small nuclei. Some epithelial cells with vacuoles, indicating slight secretory activity. In this case there is perhaps present a beginning of a change in the direction toward the development of hypotypical ovaries; but as yet the ovaries have not reached this condition. The state of the mammary gland and possibly of the ovaries suggests that an abortion has taken place a relatively short time previous to the examination. The large vacuolar corpora lutea with thick vessels, as well as the condition of the mammary gland, which shows signs of secretion, suggest such a conclusion.

*Guinea Pig No. III.—Ovaries:* Young corpora lutea. Loose central connective tissue fills the cavity. Atretic yellow vacuolar body with thick vessels (remnant of corpus luteum). Good small, medium and almost large follicles. No large follicles, but a few almost large follicles with granulosa degeneration. A number of small-medium or medium follicles lost their granulosa through a gradual process of solution and form cysts in which the egg is lying free. In other degenerating follicles of this kind connective tissue grows into the cavity. Many follicles in last stage of connective tissue atresia. *Mammary gland:* Good sized, acini with cuboidal epithelium, fibrous stroma. No mitoses. The ovaries are not yet hypotypical, but the condition of the smaller follicles indicates a beginning change in this direction.

*Guinea Pig No. IV.*—Nine days after the beginning of the experiment the animal was offered a larger quantity of food, but refused to eat it. One day later it was found in a very weak condition and it was examined. *Ovaries:* Corpora lutea vacuolar, with thick vessels; they show beginning degeneration, atretic yellow bodies (the remnants of degenerated corpora lutea). Good small and small-medium follicles. Large follicles show granulosa degeneration. Well preserved large follicles are not present. Follicles in various stages of connective tissue atresia. *Uterus:* Relatively low surface epithelium. Similar epithelium in glands. Fibrillar mucosa without mitoses. *Mammary gland:* small gland in a resting condition with marked development of

fibrous tissue. In this case the ovaries are not yet hypotypical, but the lack of good medium and large follicles indicates that some inhibition in the growth of follicles occurred, while the large follicles that had developed previously failed to mature and degenerated.

*Guinea Pig No. V.*—Weight June 30, 439 grams; July 9, 289 grams. From July 10 on a full ratio of food was given daily and the animal gained in weight. On July 21 it weighed 396 grams; it had not yet regained its original weight. On that date it was examined. *Ovaries:* Contained an atretic yellow body (remnant of a corpus luteum). Good follicles of all sizes, including good large follicles. Large follicles in granulosa degeneration and in various stages of connective tissue atresia. *Mammary gland:* Small intermediate, no mitoses. In this case the ovaries are on the whole normal; but apparently mature follicles did not develop and an ovulation had not taken place for a long period of time previous to the examination, despite the fact that the animal had regained part of its weight.

We find then in this series that hypotypical ovaries did not develop as the result of underfeeding; but in Guinea Pig Nos. II., III., and IV. we find some changes which indicate a tendency toward the development of hypotypical ovaries; either a premature atresia sets in in follicles which are only of medium size, or even smaller, or at least a retardation in the development of large follicles apparently occurred toward the end of the experiment. In all cases mature follicles were absent and ovulation was delayed. In the one animal which received a full ratio of food after a preceding period of underfeeding the follicles likewise failed to mature and ovulation failed to take place during the period in which the animal gained in weight. It is, however, possible that the delay in ovulation was not exclusively due to the undernourishment. In one case abortion had in all probability taken place previous to the examination, probably as the result of the underfeeding. In all these cases the relative and especially the absolute loss of weight had been as great as in the guinea pigs of the III. and IV. series, or it was even greater, and yet hypotypical ovaries had not developed. This result may be due to the greater weight of the animals at the beginning of

the experiment. All the guinea pigs weighed between 400 and 500 grams. These animals were therefore less affected in their general health by the lack of sufficient food than the animals in the other series. Only guinea pig No. IV. showed marked weakness. The duration of the experiment was on the average somewhat shorter than in series III. and IV., and it is possible that this factor also has something to do with the result. But it is probable that the greater weight of the animals in the beginning of the experiment is mainly responsible for the lack of the development of hypotypical ovaries.

*First Series of Experiments* (See Table IV.).—In this experiment the quantity of food given and changes in weight of the animals had not been determined with the same accuracy as in the later series. While we must consider the possibility of errors in the figures for the weight of the animals it is probable that the error on the whole is not considerable. In most cases the amount of food was increased for several days during the experiment and there was therefore a transitory gain in weight during a certain period. Animals *a*, *b*, *c* and *d* had been thyroidectomized some time previous to the beginning of the experiment; the remaining five animals had been normal.

*Guinea Pig No. a.—Ovaries:* Atretic yellow bodies (remnants of old corpora lutea). Hypotypical ovaries of second order. Hypotypical uterus. *Mammary gland:* Much gland tissue. Resting, intermediate gland.

*Guinea Pig No. b.*—March 20, weight 650 grams; April 7, 600 grams; April 30, 280 grams. *Ovaries:* Retrogressing corpora lutea, good follicles of all sizes. Large follicles with granulosa degeneration; follicles in various stages of connective tissue atresia. Not hypotypical ovaries; but no mature follicles developed and ovulation was delayed. *Uterus:* With ordinary surface epithelium and glands. Some vacuoles in surface epithelium. Fibrillar mucosa. Some cystic gland ducts at surface.

*Guinea Pig No. c.*—March 20, 350 grams; April 7, 275 grams; April 11, 175 grams. Found dead. *Ovaries:* With atretic yellow bodies. Hypotypical ovaries of first order. Hypotypical uterus.

*Guinea Pig No. d.*—Weight March 20, 500 grams. Receives

full ratio of food; but loses some weight. April 7, 475 grams; April 10, 400 grams, found dead. Had probably been sick. *Ovaries:* Vacuolar corpus luteum with thick vessels. Small medium and almost large food follicles; some small to medium follicles apparently begin to show connective tissue atresia. *Uterus:* Quiescent with follicular mucosa. *Mammary gland:* Large, resting-intermediate. In this case the loss in weight in the animal caused a condition in the ovaries which is approaching the hypotypical state; there are, however, as yet present some slmost large follicles.

TABLE IV.

I. SERIES.

| Guinea Pig. | Original Weight. | Change in Weight in Per Cent. of Original Weight. | Duration of Experiment. | Result. |
|---|---|---|---|---|
| No. *a*.. | 700 grams | −46% | 24 days | Found dead. Hypotypical ovaries of second order. No maturation of follicles. No ovulation. |
| No. *b*.. | 650 grams | −57% (In 23 days −53%) | 41 days | Killed. Not hypotypical ovaries. No mature follicles; retrogressing corpora lutea. Delayed ovulation. |
| No. *c*.. | 350 grams | −50% (In 4 days 21% loss of weight) | 22 days | Found dead. Hypotypical ovaries of first order. No new ovulation. |
| No. *d*.. | 500 grams | −20% (In 3 days 15% loss of weight) | 16 days | Imperfect control. Found dead. Receives a larger quantity of food; almost large follicles in ovaries but some small-medium follicles undergo connective tissue atresia. |
| No. *e*.. | 367 grams | Loses 100 grams (27%) in 1st two weeks, then regains weight | 38 days | Control; full ratio of food. About 4½ days after ovulation. Normal cycle took place. |
| No. *f*.. | 330 grams | −45% | 14 days | Died. Hypotypical ovaries of first order. No new ovulation. |
| No. *g*.. | About 420 grams | −58% | 17 days | Died. Hypotypical ovaries of first order. No new ovulation. |
| No. *h*.. | About 420 grams | −58% | 21 days | Hypotypical ovaries of second order. No new ovulation. |
| No. *i*.. | About 360 grams | −58% | 37 days | Hypotypical ovaries of second order. No new ovulation. |

*Guinea Pig No. e.*—Control. Weight March 24, about 365 grams. April 7, 260 grams; received full ratio. May 1, 359

grams; examined. *Ovaries:* Two young corpora lutea with capillaries, with mitoses in lutein and endothelial cells. Atretic yellow bodies. Good small and small-medium follicles; follicles in medium and late connective tissue atresia. *Uterus:* Epithelium in glands high; frequent mitoses in gland fundi. In mucosa predecidual cell layer with frequent mitoses. *Mammary gland:* Large ducts, acini with rather high cuboidal epithelium, very cellular, not fibrous stroma; probably mitoses in acini. This is an animal at a period about 4½ days after ovulation. Mature follicles developed and ovulation took place.

*Guinea Pig No. f.*—Hypotypical ovaries of first order with small, vacuolar, retrogressing corpus luteum with thick vessels. Hypotypical uterus with a few mitoses in gland ducts.

*Guinea Pig No. g.*—Hypotypical ovaries of first order; corpus luteum with vacuolar cells and dense fibrous nucleus; probably 15–20 days old. Atretic yellow bodies. Hypotypical uterus. Ovulation had taken place approximately at the time of the beginning of the experiment. The atretic yellow bodies indicate that previous to the experiment the sexual cycle had taken its normal course.

*Guinea Pig No. h.*—Weight March 24, about 420 grams. April 7, weight 220 grams. April 13, 175 grams. Killed April 14. *Ovaries:* Atretic yellow bodies (remnants of corpora lutea); small and small-medium good follicles. In the small-medium follicles atresia sets in and connective tissue grows into the cavity. A few follicles reach almost medium size and then disintegration of the granulosa and connective tissue atresia set in. Only exceptionally a good medium sized follicle present. Hypotypical uterus; some mitoses in surface epithelium. *Mammary gland:* large. Small acini with or without colloid, with low or medium-sized epithelium and partly vacuolar cells. Stroma fibrillar or fibrous. There may have been secretion in this gland previous to the beginning of the experiment.

*Guinea Pig No. i.*—Hypotypical ovaries of second order with atretic yellow bodies. Hypotypical uterus; some mitoses in surface epithelium. Guinea pigs *h* and *i* were evidently not so strongly affected by lack of food. They did not die spontaneously at the end of the experiment.

From the results of this series, we may draw the following conclusions: Of the seven animals which were subjected to the low diet, 3 showed hypotypical ovaries of the first order; 3 showed hypotypical ovaries of the second order. In all of these cases ovulation had been prevented. In one case, the ovaries were not hypotypical, although the loss in weight had been just as considerable as in other cases. In this case, however, maturation of follicles and ovulation failed to take place. If we inquire into the condition responsible for these differences in the behavior of different animals, we may suggest that it depends in all probability on differences in the general effect of underfeeding on the animals. Those animals that were alive at the end of the experiment showed a less far-going hypotypical condition than those that had just died at the time of examination. This series shows furthermore that a hypotypical condition may be produced even in a relatively old guinea pig with a considerable initial weight, provided there is a considerable loss of weight extending over a relatively long period of time and a marked influence in the general condition of the animal. If, on the other hand, an animal with a considerable initial weight is less affected generally and its strength is better preserved, throughout the experiment, merely maturation of the follicles and ovulation are prevented from taking place, but a hypotypical condition is not produced. In all those cases in which the ovaries are hypotypical the uterus is thin and shows the resting condition found also in the other series. In this series the loss of weight of the underfed guinea pigs was more considerable than in the other series and the experiment extended over a longer period of time. In the two control animals a hypotypical condition of the ovaries was not found. In one of these the cycle in ovaries and uterus took its normal course; in the other, a loss of weight had occurred, the cause of which remained obscure. While in this case the ovaries do not show a hypotypical condition, some retardation in the development of the follicles had occurred.

## Conclusions.

We used in our experiments altogether thirty-four guinea pigs, eight of which served as controls, while twenty-six were sub-

jected to insufficient feeding extending over periods varying between 33 and 6 days. In the majority of experiments the loss in weight varied between 25 per cent. and 35 per cent. of the initial weight. In some cases the loss was greater, in others less. In the controls there was usually a gain in weight during the experiment, but in two cases there was some loss and in one the loss was as much as 20 per cent. of the initial weight. This animal cannot be considered as a perfect control. In the controls the sexual cycle took its normal course and ovaries and uterus were normal in seven animals. In three of these pregnancy was present which proceeded normally. In several others ovulation occurred during the time of the experiment and the follicles developed in a healthy manner. In the one case, in which the animal lost 20 per cent. of its weight, the ovaries were not yet hypotypical, but some change was noticeable tending in that direction. Of the animals subjected to lack of a sufficient quantity of food the ovaries were hypotypical in eighteen cases. There were differences in the degree in which this condition had developed; while the ovaries of some showed a hypotypical condition of the first order, in others hypotypical ovaries of the second order were present. In the former the development of the follicles proceeded only to that stage in which the follicles were of small-medium size. At this stage atresia set in, while in the second kind a few follicles developed to medium or almost medium size; while others became already atretic at an earlier stage. In no case were large follicles produced in any of these ovaries. In three other cases changes were noticeable in the ovaries which suggested a beginning in the direction toward the development of hypotypical ovaries, while in five animals hypotypical changes were not yet noticeable, but an inhibition of ovulation had occurred owing to the fact that the large follicles failed to mature. We see then that in all the animals a failure of the follicles to mature occurred and in 69 per cent. of the animals the ovaries were hypotypical. In addition pregnancy was found in none of the animals. In one of them abortion had occurred, with certainty and in a second one with great probability, in both cases probably as a result of the underfeeding.

If we now inquire into the causes of the variability in the

effects of the underfeeding on the ovaries, our experiments suggest very strongly that in addition to the relative loss in weight two other factors may be of significance, viz., first the length of time during which the loss of weight occurs. It seems that more than six days must elapse, before hypotypical ovaries are found. And secondly, the weight of the animal at the beginning of the experiment is of importance. The more the animal approaches full size, the greater is the difficulty with which the changes in the ovaries set in. They are more readily produced in animals weighing between 300 and 400 grams than in animals weighing over 400 grams. But they can be produced in the latter. The effects on the ovaries are therefore to some extent parallel to the effects of undernourishment on the general condition of the animals. Heavier guinea pigs who suffer the same percentage in loss of weight as smaller animals are apparently very much less affected by it as far as their general health is concerned. It is probable that the greater the portion of the food intake is which the animals have to set apart for growth, the more the portion to be used for maintenance is diminished and it seems furthermore that the effect on the general health and on the condition of the ovaries depends upon the portion available for maintenance. The state of the ovaries determines the condition of the uterus, which is always in a resting, almost atrophic condition in cases in which the ovaries are hypotypical. In a similar manner the condition of the mammary gland depends upon the state of the ovaries. We see then that as a result of underfeeding there takes place an increased destruction of granulosa cells which affects even follicles of small or small-medium size. This degeneration of the granulosa cells at such an early stage of the development of the follicles does not occur in the form of a massive destruction, but here and there cells in the rows of the granulosa adjoining the cavity of the follicles are dissolved. While we may notice cells in which the nuclei undergo karyorrhexis, it is probable that other cells perish without a distinct karyorrhexis taking place. If, on the other hand, a degeneration sets in in the larger follicles in normal ovaries, a large number of granulosa cells are destroyed simultaneously and the nuclei of all these cells show karyorrhexis.

We must therefore assume that if the amount of available food

stuffs is diminished below a certain quantity, those cells perish first which are farthest removed from the source of nourishment which is furnished by the capillaries of the theca interna. But the destruction is not limited to the cells adjoining the cavity, but affects in the end the whole granulosa. We see then that under the condition of lack of proper nourishment, a condition which otherwise would occur much later, is observed at an early period of the development of the follicles, and the process of destruction in this case takes place more slowly than in the case of atresia of the large follicles. It is in the small follicles a more chronic, in the large follicles a sudden, acute process. Those granulosa cells in the small and small-medium follicles which survive for a certain time show approximately the same proliferative energy as the granulosa cells of similar follicles in normal ovaries as the investigations of L. S. N. Walsh have shown.[1] We must therefore assume that the remaining cells are fairly healthy and respond to the stimulus to multiply in a way similar to the granulosa cells in normal follicles, while those cells that are markedly affected by the lack of nourishment become dissolved. We have here to deal with a phenomenon similar to the one which we observed in the case of stationary or retrogressing tumors. In our experimental analysis of tumor growth we noticed that in tumors that had ceased to grow mitoses could still be found rather frequently during a certain period following cessation of growth. This observation led us to the conclusion that the stationary or retrogressing condition was brought about not so much through a complete cessation of growth, as through an increased destruction or solution of cells.[2] We see that in the hypotypical follicles the smallest follicles resist, while in the follicles in which a cavity begins to form processes of degeneration set in. We observe the same phenomenon in the destruction of follicles which takes place at the time of ovulation. Here also the largest follicles perish first and the smallest follicles are most resistant. We must assume either that with the growth of the follicles the granulosa cells undergo changes in their constitution, which makes them more sensitive to injurious

[1] Walsh, L. S. N., *Journal Exp. Medicine*, 1917.

[2] Loeb, Leo, *Virchow's Archiv*, 1902, CLXVII., p. 175; 1903, CLXXII., 345; also E. P. Carson White and Leo Loeb, *Centralbl. f. Bact.*, 1910, LVI., 488.

influences, or that with the growth of the follicles the nourishment becomes more difficult, and that this unfavorable state of the cells makes them more vulnerable, if injurious conditions arise. In case the second interpretation should be correct we would have to assume that the process of degeneration leads auto-katalytically to further destruction of the follicles of the remaining granulosa cells.

These observations lead furthermore to the conclusion that the formative stimuli, which call forth cell proliferation, are not identical with the food stuffs on which ultimately the life of the cells depends and which are therefore necessary for cell multiplication, a conclusion in full accord with other facts which may be discussed in another connection. Our previous observations and especially the additional observations of Walsh enable us to state the character of the formative stimulus which acts on the granulosa cells. As far as we can determine this stimulus emanates from the egg, and affects principally those granulosa cells nearest the egg, but to a less extent also the more distant cells. It is probable that this stimulus increases simultaneously with the growth of the follicles from small to medium size. In the medium-sized follicles it reaches a maximum. From now on certain unfavorable factors begin to make themselves felt with increasing severity and they lead ultimately either to a degeneration of the granulosa or to that increased differentiation which is characteristic of maturation of the follicles. Perhaps it is the difficulty in the nourishment of the granulosa—a difficulty which increases with the further enlargement of the medium follicles—which is the factor which counteracts the cumulative action of the ovum and prevents a steady increase in proliferative power of the granulosa cells, and which ultimately leads to their death. Thus may be explained the typical growth curve consisting of an ascending and a descending branch as has been described by Walsh. As we stated above, the ova in the follicles which in the hypotypical ovary undergo a premature atresia, show progressive changes. Thus the same factor that causes destruction of the granulosa cells acts on the eggs as a stimulus. In a similar manner connective tissue cells show activity, migrate into the follicular cavity and thus contribute to the atresia of follicles under conditions in which the granulosa cells perish in

consequence of the lack of proper nourishment. We may then conclude that the connective tissue is more resistant to withdrawal of food than the granulosa cells; and this is a conclusion which is borne out by the behavior of the connective tissue under other conditions. This relatively great resistance to lack of food is one of the most important characteristics of the connective tissue which leads to interesting consequences under normal and pathological conditions. While on the whole the connective tissue cells are more resistant than the granulosa cells yet we noticed that under the conditions of ill nourishment prevailing under our experimental conditions, the ingrowth of connective tissue into the follicular cavities was not rarely somewhat retarded. In such cases we see the follicle forming a cyst lined by theca interna in which the ovum lies free or almost free and in which connective tissue has not yet grown.

The atresia of follicles is comparable to a condition which in other organs is designated as cirrhosis. In the case of the atresia of follicles this cirrhotic process is a substitutive one; the stimulus which induces the connective tissue to become active consists in the loss of the granulosa cells which formerly covered the theca interna. As we pointed out on former occasions, we have reason to assume that there exist in addition stimuli of a different character which call forth cirrhotic processes.[1]

From our investigations it follows that in the guinea pig underfeeding prevents maturation of the follicles and thus causes sterility which lasts as long as the effect of the underfeeding is present in the ovary. This lack of maturation we found in all our cases. In addition we found in the large majority of those animals which had become more markedly affected by the lack of sufficient food, especially in those which had not yet reached their full size, a much farther-going deficiency in the development of the follicles, a condition of the ovaries to which we applied the name "hypotypical." The latter state is altogether incompatible with fertility. The problem that is as yet unsolved concerns the length of time during which this condition is present, and whether the ovaries resume their normal life very soon after the quantity of food has again been increased, or whether the hypotypical condition and lack of maturation

[1] Loeb, Leo, *Journ. Amer. Med. Ass'n*, 1915, LXIV., 726.

persists at least for some time after the resumption of feeding. Further investigations will have to solve this problem. Our results may perhaps also have some bearing on the condition of the ovaries during pregnancy. We have shown that in guinea pigs ovulation does not occur during pregnancy unless the corpora lutea have been extirpated. In other species however, and especially in man it seems that during pregnancy a maturation of the follicles is lacking. Our experiments suggest that this may depend upon a relative insufficiency of food available for the ovaries during pregnancy; thus an approach to a hypotypical condition of the ovaries would be produced.

## SUMMARY.

Underfeeding, if very pronounced, prevents maturation of the follicles in the ovaries of the guinea pigs in all cases and in the large majority of cases leads to the production of hypotypical ovaries in which atresia of follicles sets in before the follicles have reached medium size. Underfeeding leads to a premature solution of granulosa cells. Connective tissue is more resistant to lack of food than the granulosa. The *uterus* in cases of underfeeding is in a resting or atrophic condition. Thus underfeeding produces at least temporary sterility. Those cells farthest removed from the blood vessels suffer first as the result of underfeeding, die and become dissolved, while in those granulosa cells which remain alive the growth stimulus which in part at least emanates from the ovum causes for a short time a normal cell proliferation. Just as the underfeeding produces more pronounced general effects in younger animals, the effect on the ovaries is likewise more marked in younger animals. Through underfeeding it is, however, possible to produce a hypotypical condition even in old guinea pigs. In those animals which had been pregnant at the beginning of the experiment underfeeding led to abortion.

There exists a noteworthy analogy in the relation of cell proliferation to cell destruction in the hypotypical ovaries and in stationary or retrogressing tumors.

Our results emphasize the distinction between food stuffs and proliferative cell stimuli and they may in addition throw light on the condition of the ovaries during pregnancy.

# EXPERIMENTS WITH FEEDING THYMUS GLANDS TO FROG LARVÆ.

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## INTRODUCTION.

In 1912, J. F. Gudernatsch published an account of his investigation concerning the effects of feeding various glands of internal secretion to frog larvæ. Among other interesting results observed, was the significant one, that fresh thymus gland, when fed to tadpoles, stimulates the growth processes of these animals and at the same time inhibits the onset of metamorphosis.

In a later communication, January, 1914, this author repeated his previous experiments with thymus feeding, and confirmed his earlier results. For an understanding of the relation between growth and the secretory products of the thymus gland, the importance of the results obtained by this investigator warranted a repetition of his work, which, so far as the writer is aware, has never been questioned. It was with this end in view that the following experiment was undertaken.

## LITERATURE.

The experimental researches upon the thymus gland may roughly be divided into two groups: those dealing with the relation between the thymus and bodily growth, and those concerned with the correlation in function of the thymus and sex glands. The literature dealing with the former problem is more extensive than that concerned with the latter.

### EFFECTS OF EXTIRPATION UPON GROWTH.

One of the first investigators of this problem was Carbone; this experimenter extirpated the thymus of rabbits, and one dog, and found that the animals did not differ in their development from control animals of the same litter.

Another investigator, Ghika, removed the thymus from eight

cats and thirteen rabbits; he found that in the first few days there was emaciation and arrest of development which lasted for weeks, although, in some of the animals, the losses were afterwards made good.

Cozzolino removed the thymus from young rabbits weighing 300–400 gm., and in the first two months after the operation was unable to detect any change in their condition.

In a later communication, however, he described crookedness of the legs, arrest of growth, and progressive cachexia in one animal upon which he had operated three months previously.

S. Vicent extirpated the thymus of guinea pigs and did not observe any changes in their condition.

Paton and Goodall found that extirpation of the thymus produced no effect upon the general growth and development of new born guinea pigs.

R. Fischel extirpated the thymus of goats, rabbits and dogs, and obtained negative results. He concluded from his experiment, that thymectomy has no effect upon the development of the subject.

The work of this author has been severely criticized by Basch, who claims that the experimental conditions were inappropriate.

Basch extirpated the thymus of suckling dogs and obtained, striking changes in the growth of his thymectomized animals. The most important growth changes were those affecting the skeleton.

U. Soli found that thymectomy was followed in young rabbits by changes in the development of the skeleton, these changes affecting the hollow, long bones and ribs. This author failed to confirm the results obtained by Basch, with guinea pigs, in which thymectomy is a comparatively simple operation. This would seem to indicate that the symptom complex, observed by Basch following thymectomy in his animals, was probably due to post-operative changes and not connected with the thymus, because in those animals used by Basch, thymectomy is a very severe operation.

Halnan and Marshall extirpated the thymus of young guinea pigs, and found that removal had no influence upon the growth of these animals.

Pappenheimer extirpated the thymus of young albino rats and found that removal had no effect upon body growth and development.

Adler extirpated the thymus of tadpoles and found that these animals suffered no ill effects from the operation. Growth and development was unaffected.

(The work of Gudernatsch has been noted.)

This rather extensive review of the literature was thought justifiable in view of the discrepancy in the results obtained by the various investigators.

## Material and Observations.

The following experiment began May 3, 1915, and was completed July 29, 1916. The work in 1915 dealt with the effect of feeding thymus powder to tadpoles, the work for 1916 was concerned with those experiments in which only the fresh gland was employed as food. I shall discuss the work dealing with the fresh gland first, and devote the latter part of this paper to feeding experiments with thymus powder.

## Feeding Fresh Thymus Gland.

This work began April 12, 1916. April 2, the eggs of *Rana pipiens* were gathered from a group of shallow pools near the University campus at Lawrence, Kansas, and allowed to develop in the laboratory. One group of eggs was hastened considerably in development by keeping them in a warm room. When the young larvæ had emerged from their gelatinous egg capsules and developed to the free feeding stage, they were placed in glass bowls ten inches in circumference by four inches deep. The tadpoles were covered with ordinary tap water which was changed daily.

When the larvæ had attained a length of nearly 13 mm., one hundred and fifty were selected, and divided into two groups of seventy-five each; one lot was destined for thymus feeding, the other for control purposes. These two lots of seventy-five animals were then subdivided into lots of ten and placed in glass bowls, one lot to a bowl. Both control and experimental larvæ were reared under identical conditions as regards temperature, light and water supply.

A second series of larvæ, one hundred in number and averaging 10 mm. in length, were selected and treated the same as the series above. Fifty larvæ were for thymus feeding and fifty for control purposes. These larvæ were from a batch of eggs gathered a few days later than the larvæ of the first series. Hence, throughout the following experiment, the series averaging nearly 13 mm. in length when started upon the thymus feeding I shall designate as Thymus No. 1, the series averaging 10 mm., at the beginning of the work, as Thymus No. 2.

TABLE I.

APRIL 12.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 12 | 11 | 10.5 | 10 |
| 12.5 | 13 | 11 | 9 |
| 11.5 | 12 | 9 | 11 |
| 13 | 11 | 10 | 9.5 |
| 13 | 12 | 10.5 | 10.5 |
| 13.5 | 13 | 10 | 10 |
| 12.5 | 13.5 | 9 | 9 |
| 11 | 13 | 11 | 9.5 |
| 12 | 12.5 | 9.5 | 9 |
| 12.5 | 11.5 | 10.5 | 10.5 |
| 11 | 14 | 10 | 11 |
| 11 | 13 | 11 | 11.5 |
| 13 | 13 | 10 | 10.5 |
| 13.5 | 14 | 10.5 | 11.5 |
| 12.5 | 13.5 | 9.5 | 11 |
| 11.5 | 11.5 | 9 | 11.5 |
| 11 | 12.5 | 11 | 10 |
| 12 | 13 | 9.5 | 10.5 |
| 13 | 13.5 | 10 | 9.5 |
| 13 | 13 | 10.5 | 10 |
| Av. len.....12.25 | 12.6+ | 10.1 | 10.25 |

The experimental larvæ of both series were each day fed finely shredded bits of the fresh thymus glands of calves 3–12 months of age. Only the glands of young animals were used. The glands were obtained from a nearby slaughter-house, and extirpated by myself.

The control animals were fed fresh beef liver each day. Care was taken to give as nearly as possible the same amount of protein material to the animals in each container and of each

group. Both thymus- and beef-fed larvæ ate greedily the food given them.

At the beginning of the experiment twenty larvæ of both the control and thymus-fed groups of larvæ were measured with a millimeter rule, and the average length computed. This seemed a satisfactory method of procedure, considering that all of the larvæ came from the same batch of eggs, and consequently were of the same age.

A very convenient method of measuring the young larvæ is to lay them upon moistened blotting paper. The moisture in the paper prevents injury to the tail and at the same time the larvæ are unable to lash about.

Table I represents the measurements of twenty larvæ of both control and thymus-fed groups April 12, the day the experiment began.

None of the larvæ measured revealed any indications of limb development when examined under the microscope.

### Observations of April 20.

When examined upon this date no observable differences in color or activity of the control and thymus-fed larvæ were noticed. None of the larvæ possessed limb buds. Considerable growth, however, had occurred in the larvæ of both groups, but in regard to length and body size, both control and thymus-fed animals were about equal, as Table II indicates.

### Observations of April 29.

A careful examination upon this date, of the larvæ of both thymus-fed and control groups failed to reveal any differences in pigmentation, activity, or mortality among the animals. Upon examination under the microscope, tiny limb buds of the posterior extremities were observed on those larvæ of the control group measuring 25 mm. or over. Limb buds were observed on seven of the thymus-fed larvæ measuring 27 mm. and over, in the thymus No. 1 group.

The limb buds of the larvæ at this early stage appeared as two round, blunt projections somewhat anterior and lateral to the anal opening.

## TABLE II.

APRIL 20.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 18 | 18 | 14 | 15 |
| 19 | 18.5 | 15 | 15.5 |
| 18.5 | 19 | 14.5 | 15.5 |
| 18.5 | 19 | 14 | 14.5 |
| 18 | 19.5 | 15.5 | 16 |
| 20.5 | 19.5 | 16 | 14 |
| 19.5 | 18.5 | 16 | 14.5 |
| 20 | 17.5 | 15 | 15 |
| 18.5 | 18.5 | 15.5 | 14.5 |
| 18 | 17 | 14.5 | 16 |
| 19 | 18 | 16 | 16.5 |
| 19.5 | 19.5 | 15 | 15 |
| 18 | 19.5 | 15 | 15.5 |
| 18 | 18.5 | 14.5 | 16 |
| 17.5 | 18 | 14.5 | 15 |
| 18.5 | 20 | 14 | 15.5 |
| 17.5 | 17.5 | 16 | 15.5 |
| 19 | 18 | 15.5 | 16 |
| 19.5 | 18.5 | 15 | 14.5 |
| 18 | 20 | 15.5 | 15.5 |
| Av. len....18.65 | 18.62+ | 15.05 | 15.22+ |

The length of the larvæ in both control and thymus-fed animals was about the same though several slight discrepancies are revealed in Table III. The thymus No. 1 group shows a slight acceleration in growth (average length) when compared with their liver-fed controls. This acceleration was not shown by the thymus No. 2 group. These small growth differences are shown in Table III.

The slight difference in the length of the thymus-fed and control animals may possibly be due to the fact that thymus tissue is somewhat more compact than liver tissue and probably presented greater difficulty for the tadpole in eating. Both kinds of tissue were, however, greedily devoured by the larvæ.

### OBSERVATIONS OF MAY 4.

Very faint differences in pigmentation were noticed between the larvæ of the thymus-fed and control groups. The thymus-fed animals were slightly darker than their controls which had a reddish tinge. As regards activity, body-shape and mortality,

## TABLE III.

APRIL 29.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 26 l.b. | 25.5 l.b. | 23 | 22 |
| 25 " | 26 " | 22.5 | 23.5 |
| 24.5 | 25 " | 21.5 | 23 |
| 28 " | 25.5 " | 22.5 | 22.5 |
| 28 " | 25 " | 22 | 22 |
| 25 | 25.5 " | 23.5 | 21.5 |
| 29 " | 30 " | 22 | 21.5 |
| 25 | 25.5 " | 21 | 22.5 |
| 26.5 | 24.5 " | 21.5 | 22.5 |
| 27 " | 29 " | 23 | 22 |
| 27 " | 23.5 | 23.5 | 23.5 |
| 25 | 24 | 22.5 | 23 |
| 25 | 23 | 22 | 23.5 |
| 24.5 | 24.5 | 22 | 22.5 |
| 24.5 | 23.5 | 23.5 | 22 |
| 26 " | 25 " | 23 | 23.5 |
| 25 | 23.5 | 22 | 22 |
| 27 | 24 | 23.5 | 22 |
| 26 | 24 | 22.5 | 23.5 |
| 25.5 | 23 | 22 | 22.5 |
| Av. len....25.97 | 24.97[1] | 22.45 | 22.55 |

no differences between the animals of the two groups were observed. When examined under the microscope, both the control and thymus-fed larvæ had hind limb buds. The limbs had not as yet differentiated into their two primary divisions, nor had the toe points developed. The limb buds of both groups of animals appeared to be of nearly the same size and in the same stage of development. The thymus No. 2 group of larvæ had by this time outstripped the thymus No. 1 group in growth.

The table below shows that the control animals of the thymus No. 1 group averaged, upon this date, somewhat greater length than any of the thymus-fed larvæ. The animals of the thymus No. 2 group were somewhat larger than their controls.

### OBSERVATIONS OF MAY 8.

The thymus-fed larvæ upon this date were distinctly darker in color than their controls; no other differences were observed in the two groups of tadpoles. Both controls and thymus-fed

[1] l.b. = limb buds.

## TABLE IV.

MAY 4.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 28 l.b. | 29 l.b. | 31 l.b. | 26 l.b. |
| 30 " | 31 " | 33 " | 33.5 " |
| 29 " | 37.5 " | 34.5 " | 34 " |
| 28.5 " | 34.5 " | 32.5 " | 25.5 " |
| 27.5 " | 33 " | 33 " | 32.5 " |
| 32 " | 30.5 " | 27.5 " | 32 " |
| 34 " | 28 " | 30.5 " | 30 " |
| 28.5 " | 37 " | 30 " | 26.5 " |
| 34.5 " | 30 " | 35 " | 33 " |
| 32 " | 33.5 " | 32 " | 29 " |
| 28.5 " | 30.5 " | 33.5 " | 25.5 " |
| 29.5 " | 37 " | 37 " | 33 " |
| 34 " | 33.5 " | 31.5 " | 30 " |
| 30 " | 34.5 " | 30.5 " | 29 " |
| 26.5 " | 32.5 " | 30 " | 25.5 " |
| 33 " | 34.5 " | 31.5 " | 29 " |
| 31 " | 34 " | 32 " | 27.5 " |
| 27.5 " | 32 " | 32.5 " | 30 " |
| 30 " | 30.5 " | 34 " | 31 " |
| 34 " | 33 " | 33 " | 32 " |
| Av. len....30.42 | 32.8 | 32.22 | 29.72 |

larvæ possessed hind limb buds; these were plainly visible without the aid of a hand lens. The larvæ of both groups of animals had increased in size considerably but the average length of the larvæ of each group varied but little, and such variations are negligible. This is clearly indicated in Table V.

### OBSERVATIONS OF MAY 13.

When examined the larvæ of both control and thymus-fed groups had increased in length considerably. All of the tadpoles of both groups had developed hind limb buds and most of the limb buds of the thymus-fed larvæ had differentiated into their two primary divisions and had developed the toe points. The controls seemed somewhat behind the thymus-fed larvæ in respect to the length of the limbs. The pigmentation of the experimental animals was darker than the liver-fed controls.

In other respects the larvæ of the two groups appeared the same. Table VI indicates that the larvæ of all groups averaged nearly the same length. The differences are negligible.

## TABLE V.

MAY 8.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 31.5 l.b. | 31.5 l.b. | 31.5 l.b. | 35 l.b. |
| 30.5 " | 37 " | 33 " | 31.5 " |
| 31 " | 34 " | 34 " | 32 " |
| 30.5 " | 37 " | 32.5 " | 33.5 " |
| 30 " | 34 " | 34 " | 30.5 " |
| 36.5 " | 33 " | 35 " | 30 " |
| 40.5 " | 37.5 " | 34 " | 35.5 " |
| 29 " | 38 " | 33 " | 34 " |
| 30 " | 33 " | 33.5 " | 32.5 " |
| 33.5 " | 30.5 " | 32 " | 30 " |
| 33 " | 37 " | 33 " | 32 " |
| 32 " | 33 " | 37 " | 33 " |
| 30.5 " | 34.5 " | 34 " | 31.5 " |
| 33 " | 32 " | 33 " | 30.5 " |
| 29.5 " | 33 " | 30.5 " | 29.5 " |
| 29 " | 31.5 " | 35.5 " | 31.5 " |
| 30.5 " | 33 " | 32 " | 32 " |
| 33.5 " | 34 " | 31.5 " | 32.5 " |
| 32.5 " | 32 " | 33 " | 33 " |
| 34 " | 31.5 " | 30.5 " | 33 " |
| Av. len....32.02 | 33.85 | 33.12 | 32.15 |

## TABLE VI.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 37.5 l.b. p.d.[1] | 37 l.b. | 40 l.b. | 44 l.b. p.d. |
| 44 " p.d. | 36.5 " | 45 " p.d. | 45 " p.d. |
| 37 " | 42 " p.d. | 37 " | 39.5 " |
| 33.5 " | 33.5 " | 37.5 " p.d. | 40 " |
| 36.5 " | 40 " p.d. | 35 " | 33.5 " |
| 33 " | 35.5 " | 33.5 " | 45 " p.d. |
| 35.5 " | 35 " | 33.5 " | 40 " p.d. |
| 32 " | 37 " | 32 " | 35.5 " |
| 35 " | 38 " p.d. | 35.5 " | 43 " p.d. |
| 32.5 " | 37 " | 40 " p.d. | 38 " |
| 35.5 " | 40 " p.d. | 40 " | 35.5 " |
| 42 " p.d. | 48 " | 41.5 " p.d. | 39 " |
| 40 " | 35.5 " | 37 " | 38 " |
| 42 " p.d. | 33.5 " | 38 " | 42 " p.d. |
| 43 " p.d. | 35 " | 34.5 " | 42.5 " p.d. |
| 36.5 " | 34 " | 37.5 " p.d. | 41 " p.d. |
| 37 " p.d. | 35.5 " | 35 " | 35.5 " |
| 35.5 " | 37 " | 34.5 " | 34.5 " |
| 34 " | 38 " p.d. | 37 " p.d. | 39 " |
| 39 " p.d. | 36.5 " | 35 " | 37 " |
| Av. len. 37.05 | 37.22+ | 36.95 | 39.37+ |

[1] The letters p.d. indicate that the limb buds have differentiated into their two primary divisions.

### Observations of May 20.

Examination of the larvæ at this date revealed no difference between the animals of the control and thymus-fed groups as regards pigmentation, body shape, size or activity. The limb buds of both groups had by this time differentiated into their two primary divisions, and had well developed toe points. The size of the limbs of both groups appeared to be the same. The dark pigmentation of the thymus-fed larvæ had gradually faded out, and was no longer visible on May 20. Table VII shows the average lengths of the animals of the experimental and control groups. The average length is nearly the same in all groups. The greatest difference in length between the thymus-fed and control larvæ was about two millimeters.

### Table VII.

May 20.

| Length. | | | |
|---|---|---|---|
| Thymus No. 1, Mm. | Control of Thymus No. 1, Mm. | Thymus No. 2, Mm. | Control of Thymus No. 2, Mm. |
| 46 l.b. p.d. | 45 l.b. p.d. | 44 l.b. p.d. | 41 l.b. p.d. |
| 45 " " | 45 " " | 47 " " | 42.5 " " |
| 38 " " | 47 " " | 39 " | 44 " " |
| 46 " " | 45 " " | 43 " " | 39 " |
| 37.5 " " | 35.5 " " | 41.5 " " | 38.5 " |
| 39 " " | 37 " " | 40.5 " " | 41.5 " " |
| 48.5 " " | 34.5 " " | 45.5 " " | 39 " " |
| 37 " " | 44 " " | 42 " " | 40.5 " " |
| 41 " " | 46 " " | 43 " " | 40.5 " " |
| 39 " " | 37.5 " " | 47 " " | 41 " " |
| 39 " | 44 " " | 46.5 " " | 39 " " |
| 42 " | 37.5 " " | 44.5 " " | 41.5 " " |
| 40 " " | 46 " " | 38.5 " | 43 " " |
| 44 " | 35.5 " " | 40.5 " " | 44 " " |
| 37.5 " " | 46 " " | 39 " | 40.5 " " |
| 39 " " | 44 " " | 38 " | 41 " " |
| 38 " " | 43 " " | 44 " " | 39.5 " |
| 39.5 " " | 37.5 " " | 42 " " | 41.5 " " |
| 40.5 " " | 38 " " | 41 " | 38.5 " |
| 41 " " | 39.5 " " | 39 " | 40 " " |
| Av. len. 40.8 | 41.37 | 42.27 | 40.8 |

All larvæ which had developed the two primary divisions of the legs in this series, had well-developed toe points.

The larvæ were not measured again until June 8, eighteen days from the date of previous measurement. The larvæ had

grown so large that it was considered best to avoid handling them, as much as possible. During the eighteen-day interval only four tadpoles died. One was a control and the other three were thymus-fed animals. The four larvæ became bubbly, *i. e.*, air vacuoles appeared in the region of the lungs under the skin. The larvæ came to the surface and floated on their backs; death usually resulted within a few days. The following table indicates the measurements of the larvæ.

TABLE VIII.

OBSERVATIONS JUNE 8.

| Measurements of the Larvæ. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Thymus No. 1, Mm. | | Control of Thymus No. 1, Mm. | | Thymus No. 2, Mm. | | Control of Thymus No. 2, Mm. | |
| 50 | Legs 3.5 | 54.5 | Legs 6.5 | 47.5 | Legs 3 | 50 | Legs 2.5 |
| 47 | " 3 | 56 | " 10 | 57 | " 4 | 50 | " 2.5 |
| 57 | " 8 | 50.5 | " 9 | 55 | " 4 | 50.5 | " 2 |
| 49 | " 2 | 60 | " 10 | 48 | " 3 | 55.5 | " 3 |
| 60 | " 8 | 45.5 | " 2 | 44.5 | " 2 | 55 | " 4.5 |
| 52 | " 3 | 43.5 | " 8.5 | 60 | " 10 | 54.5 | " 6.5 |
| 48 | " 2.5 | 58 | " 8 | 55 | " 4.5 | 51.5 | " 2 |
| 55 | " 2 | 58 | " 10.5 | 42.5 | " 2 | 53 | " 3.5 |
| 45 | " 2.5 | 57 | " 6 | 50 | " 3 | 47.5 | " 2 |
| 58 | " 2 | 60 | " 7 | 53 | " 3.5 | 55 | " 4 |
| 49 | " 2.5 | 56 | " 5.5 | 41 | " 2 | 45.5 | " 3 |
| 60 | " 3 | 48 | " 3 | 58 | " 7.5 | 53.5 | " 5.5 |
| 51 | " 4 | 52 | " 4 | 50.5 | " 5 | 53 | " 5 |
| 53 | " 3 | 54 | " 4 | 58 | " 5 | 58.5 | " 9 |
| 58 | " 6.5 | 45 | " 2.5 | 50 | " 2 | 56 | " 6.5 |
| 57 | " 9 | 59 | " 3.5 | 55 | " 3.5 | 52 | " 4 |
| 55 | " 6 | 48 | " 2 | 55 | " 4 | 45.5 | " 2.5 |
| 50 | " 5 | 49.5 | " 2.5 | 60 | " 8 | 49 | " 2.5 |
| 56 | " 5.5 | 51.5 | " 3 | 50 | " 2 | 51.5 | " 3 |
| 60 | " 6 | 53 | " 4 | 62 | " 11 | 53 | " 5.5 |
| Av. len. 53.95 | 4.35 | 52.95 | 5.57+ | 52.6 | 4.45 | 52. | 3.95 |

## OBSERVATIONS OF JUNE 8.

The larvæ of both control and thymus-fed cultures had grown considerably in the eighteen-day interval between the observations of May 20 and June 8. All of the tadpoles of both groups possessed well-developed limbs, some measuring 11 mm. in length. The limbs of the thymus-fed animals averaged in length about the same as the controls; this was true also of body length, as a glance at Table VIII. will show.

In regard to pigmentation, activity and mortality among the

animals of the two groups, practically no differences were observed. The forelimbs of the larvæ had not, as yet, broken through the skin, but showed as slight bulges or swellings under the skin of the pectoral region. The growth of all the larvæ had been retarded, no doubt, by unseasonably chilly weather.

June 10, all of the larvæ of both thymus and liver-fed cultures, on account of their increased size, were removed from their containers and placed in very much larger basins. The new containers were especially constructed sinks, measuring 30 × 18 × 6 inches. Thirty larvæ were placed in each sink, which were so arranged that fresh, well-oxygenated water flowed through them constantly.

By June 20, two of the control larvæ, and one of the thymus-fed group, were in advanced stages of metamorphosis. The forelimbs appeared and the tail was reabsorbed. June 26, these larvæ had completely metamorphosed. From this date on, metamorphic changes appeared in almost all of the larvæ, both of the control and thymus-fed groups. By July 3, six thymus-fed and eight liver-fed larvæ had metamorphosed, and by July 12, most of the larvæ had already metamorphosed, or else were in advanced stages of the process.

The animals were all killed, and their gonads preserved for microscopic examination. Macroscopic examination of the germ glands of both thymus-fed and control animals revealed no observable differences either as regards the size of the gland or proportion of the sexes.

The gonads were fixed in Flemming's fluid and sectioned at a thickness of 7.5 μ. The sections were stained with iron alum hæmatoxylin, counter stained with congo red. Only four of the gonads were examined microscopically, two from thymus-fed and two from control larvæ.

Microscopic examination of the four gonads revealed nothing of interest. Three of the gonads were from female larvæ, and contained young oöcytes undergoing growth. The single male gland contained only spermatogonia and was of normal size. The thymus feeding appeared to have had no effect upon the growth and development of the gonads and the germ cells.

We may say, then, in the light of this experiment, that feeding

fresh thymus gland to the larvæ of *Rana pipiens* has no effect upon the growth and metamorphosis of these animals, and the gonads appear to be unaffected by such feeding.

PART II. EXPERIMENTS WITH FEEDING THYMUS POWDER.

The following experiment was started May 3, 1915: The larvæ of *Rana pipiens* were used for the first half of the work. These larvæ had been reared from the egg in the laboratory and were all from one batch of eggs. The feeding work was begun when the tadpoles averaged 28 mm. in length. Most of the larvæ had tiny limb buds of the posterior extremities, at this time, though, as yet, the buds had not differentiated into their two primary divisions. Two series of animals were used, one for feeding work, the other for controls. Both groups were kept under uniform conditions of temperature, light, and water supply. Finger bowls were used as containers, five larvæ to a bowl. Tap water was used to cover them and was changed daily.

The animals of the control group were, each day, fed fresh water algæ, and dried flour paste. This same food was also given to the thymus-fed larvæ, though in smaller amounts in order to obviate any possibility of inanition.

The thymus used was the desiccated glands of sheep prepared by Armour & Co. This powder was mixed with wheat flour in the proportions of three grams of thymus to five grams of flour, enough water was added to make a thick paste which was allowed to dry at room temperature. Pieces of the dried thymus paste about the size of one's thumb nail were finely crumbled and fed to the larvæ. The mixture seemed palatable to the tadpoles for they ate it greedily. The same amount of paste was given each day.

The control animals grew rapidly, and in a few days the limb buds had differentiated into small legs. The development of the thymus-fed larvæ was somewhat slower and, in regard to length, were two or three millimeters behind their controls. On the whole, however, growth in the two series of larvæ was approximately equal, when the fact is considered that the thymus-fed larvæ received less algæ than the controls.

The limbs of the thymus-fed tadpoles differentiated somewhat

more slowly than the limbs of the controls, and appeared to be smaller in size. There were no observable differences in the pigmentation of the two groups.

This series of larvæ was kept until June 3, just one month from the date the thymus feeding began. They were measured on this date, the control larvæ averaging 39.5 mm., the thymus-fed larvæ 37.5. The tadpoles were then killed and preserved for miscroscopic examination. It is evident that the thymus feeding did not accelerate growth in the animals of this group. The two millimeters difference between the average length of the control group and the average length of the thymus-fed group is, in animals so variable in their growth capacities as frog larvæ, negligible.

On May 9, a second series of frog larvæ were started upon the thymus diet, appropriately controlled. The controls and thymus-fed larvæ of this series averaged 35 mm. All of the larvæ had hind legs though they were very small, and in most of them the toe points were just appearing. These animals were fed the same kind, and amount of food, as the first series received.

The thymus feeding continued for eighteen days, during this period both controls and thymus-fed larvæ grew rapidly, but as recorded for the first series, the controls differentiated limbs faster.

The length of the two groups of tadpoles was approximately equal, both groups averaging 41.5 mm. eighteen days after the experiment began.

Two other series of larvæ were started later, but the tadpoles were too far along in metamorphosis. No results worth recording were obtained.

August, 1915, I obtained some larvæ of the common bull-frog, *Rana catesbiana*. When found, these tadpoles averaged 32 mm. in length. All except six were used for another experiment. The six remaining larvæ were divided into lots of three each, one for controls, the other for thymus feeding. The nature of the food, and amount fed was the same as that recorded for earlier experiments.

For twenty-one days, this series of tadpoles was fed thymus, and at the end of that period, there was no appreciable difference

between the controls and thymus-fed animals either in regard to body length, shape or stage of development. Before the experiment was discontinued the larvæ of both groups of animals were measured. The controls measured in length 41 mm., the thymus-fed animals 39.5 mm.

In this series of larvæ, as in the two previous series, thymus feeding certainly did not accelerate growth.

On November 1, another set of *Rana catesbiana* larvæ were obtained, and all larvæ of 75 mm. in length were selected for thymus feeding. There were twelve 75 mm. larvæ. They were divided into two groups of six each for controls and thymus feeding purposes. The diet of both groups was similar to that given to the larvæ of the previous series.

November 10 the animals of both control and thymus-fed groups were measured. The controls averaged 83 mm. in length; the thymus-fed larvæ 85 mm. The limb buds of both groups were very small. No other differences in the two groups were noticed.

December 9, the larvæ of both groups were again measured. The controls averaged 89.5 mm. The thymus-fed larvæ averaged 90.5 mm. Pigmentation, body shape, were the same in the animals of both groups.

December 23, the animals of both control and thymus-fed groups were again measured. The controls averaged in length 92.5 mm., the thymus-fed larvæ 93 mm. The limb buds of both groups appeared to be of about the same size and stage of development.

The experiment was discontinued at this time.

It was observed in this series that the thymus-fed larvæ averaged slightly more in length than the controls. The differences, however, are very small.

A last series of feeding experiments with powdered thymus was started November 17, 1915. Eighty small tadpoles (species not determined), averaging 17 mm. in length and without limb buds, were brought to the laboratory on this date. Thirty were selected and treated similarly to those of the earlier experiments. These animals were fed thymus from November 17 until December 7. Upon this date the tadpoles of both control and thymus-

fed groups were measured. The controls averaged 29.5 mm., the thymus-fed larvæ 30 mm. The disparity in the average lengths of the two series is negligible. The fact to be noticed is that both experimental and control larvæ grew considerably and that both groups possessed tiny limb buds on December 7, when the experiment was discontinued.

### Summary of Thymus Powder Experiment.

To sum up the results obtained in the experiments just recorded it may be said that feeding powdered thymus glands to amphibian larvæ of the species *Rana pipiens*, *Rana catesbiana* and *Rana* ——— (species undetermined) does not accelerate growth beyond the normal rate. Some of the observations recorded do, however, indicate that thymus feeding may have a slight inhibitory effect upon limb development. However, in animals so extremely variable in their growth capacities as frog larvæ, to attribute such a slight retardation in limb development to the effects of thymus feeding would be rather unsafe. Aside from this extremely slight retardation in limb development of the thymus-fed larvæ, no results were obtained which would indicate that the feeding of powdered thymus gland to amphibian larvæ has any effect upon growth.

### Summary and Conclusion.

To sum up the results of this experiment, feeding thymus gland, either in the fresh state or in the powdered form, to frog larvæ of the species *Rana pipiens*, *catesbiana*, and one undetermined species, does not accelerate the growth processes, or retard the metamorphosis of these animals. The tadpoles seem to develop normally in every respect. The gonads do not appear to be effected by thymus feeding.

These results are at variance with the findings of Gudernatsch. It may be, however, that the species of frog used by this investigator reacts differently to thymus feeding than does *Rana pipiens* and *catesbiana*, thus accounting for the disparity of the results obtained by this investigator and myself.

One point, however, must not be overlooked in this connection, *i. e.*, that the variability of the growth rate in frog larvæ is

very great, even in larvæ reared from the same batch of eggs and under similar conditions. Very slight environmental changes are sufficient to bring about fluctuations of the processes of growth in these animals, and even when the changes are reduced to a minimum individual variations are great. I have seen tadpoles of the same age, from the same batch of eggs, reared in the same bowl, that varied as much as 20 mm. in length in five weeks' time. I quote this for what it may be worth, but it serves to indicate the complexity of the factors governing the growth of amphibian larvæ. I cannot but think that the results obtained by Professor Gudernatsch are attributable, in part at least, to some other factor besides thymus feeding.

In regard to the feeding experiments with thymus gland, either in the fresh state or powdered form, one point should not be overlooked, viz., that the growth-accelerating principles of the gland, if there be such, may undergo disintegration or suffer a change by contact with the digestive enzymes in the alimentary tract. Feeding experiments with thyroid glands show that no such changes occur, but possibly the same is not true of the thymus.

In conclusion, I wish to acknowledge my indebtedness to Dr. B. M. Allen, the University of Kansas, for the time and encouragement he has given me in my work.

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